Combination therapy with Nivolumab and PD-L1/IDO peptide vaccine to patients with metastatic malignant melanoma

A phase I/II trial

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The study will be performed as described in this protocol and in accordance with regulatory requirements and Good Clinical Practice. It will be monitored by the GCP unit of the Copenhagen University Hospital and can be subject for external audition as quality assessment of the GCP unit. The investigator allows direct access to source data and documents including medical records of participants, for inspection by the relevant authorities, being the Danish Medicines Agency, GCP units and/or other health authorities.

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Abbreviations

ALAT	P-alanine-aminotransferase
ANC	Absolute Neutrophilic Count
ASAT	P-aspartattransaminase
CCIT	Center for Cancer Immune Therapy
CNS	Central Nerve System
CR	Complete Response
CRR	Complete Response Rate
CRF	Case Report Form
CRP	C - reactive protein
CT	Computer tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTL	Cytotoxic T lymphocyte
CTLA-4	Cytotoxic T-Lymphocyte Antigen 4
DMSO	Dimethylsulfoxid
EMA	European Medicines Agency
ECOG	Eastern Cooperative Oncology Group
MHC	Human Leukocyte Antigen
IDO	Indolemamine-pyrrole 2, 3-dioxygenase
IDO long	Indolemamine-pyrrole 2, 3-dioxygenase long
IgG	Immunoglobulin G
IL-2	Interleukin 2
INR	International Normalized Ratio
irRC	Immune Related Response Criteria
LDH	Lactate-dehydrogenase
MART	Melanoma Antigen Recognized by T cells
MDSC	Myeloid-derived Suppressor Cell
MHC	Major Histocompatibility complex
MM	Malignant melanoma
MR	Magnetic resonance
NSCLC	Non-Small Cell lung cancer
ORR	Objective Response Rate
OS	Overall survival
PBS	Phosphate buffered saline
PD-1	Programmed cell death 1
PD-L1	programmed cell death ligand 1
PD-L1 long1	programmed cell death ligand 1 long1
PD-L2	programmed cell death ligand 2
PERCIST	PET Response Criteria in Solid Tumors

PET	Positron Emissions Tomography
RECIST	Response Evaluation Criteria in Solid Tumors
PR	Partial Response
SD	Stabil Disease
S-HCG	serum-Choriogonadotropin
SKILs	Skin Infiltrating Lymphocytes
SOP	Standard operating procedure
TAA	Tumor Associated Antigens
TADC	Tumor Associated Dendritic Cells
TNM	Tumor-Node-Metastases
Treg	Regulatory T cells
UG-team	Urologisk-Gynækologisk team

The peptides mentioned in the protocol: (same peptide has different names)

IO101	→ IDO short
IO102	→ IDO long
IO103	→ PD-L1 long1

Protocol synopsis

1.1 Objectives and endpoints

1 The primary objective is to assess tolerability and safety of the peptide vaccine containing the peptides Indolemamine 2,3-dioxygenase long (IDO long) and programmed death ligand-1 long1 (PD-L1 long1) with Montanide ISA 51 as adjuvant to patients with metastatic malignant melanoma (MM) in combination with the immune checkpoint blocking antibody Nivolumab. The endpoint is adverse events (AE) assessed by CTCAE 4.0.

The secondary objective is to evaluate the immune response before, during and after treatment. The endpoint is responses of PD-L1 and IDO specific T cells measured by ELISPOT and cytokine release assays. The tertiary objective is to evaluate the clinical efficacy of the treatment. The endpoints will be objective response (OR), progression free survival (PFS) and overall survival (OS).

1.2 Background

MM is the 5th most common cancer in Western Europe. Contrary to other solid tumors which primarily affect elderly people, MM is known to affect younger and middle-aged people. The incidence has increased over the last 15 years with about 3-5 % per year. In Europa alone, 20.000 deaths in 2008 were caused by MM.[1]

While traditional oncological treatments such as chemotherapy and radiation therapy have a poor effect on MM,[2] immunotherapy has shown very promising results. One of the novel immunotherapeutic treatments, Nivolumab, was in 2015 approved as first-line standard therapy to patients with MM.

Cancer cells are naturally attacked by cells of the immune system, among others the cytotoxic T cells. Cancer cells can induce a state of tolerance whereby they can escape from the immune attack.

This escape is brought about by many different mechanisms; some of the most important are through overexpression of the programmed cell death 1 (PD-1)/PD-L1 molecules and the metabolic enzyme IDO.

The PD-1 receptor is expressed on T cells amongst other cells. The blocking of PD-1 on T cells with PD-1 blocking antibodies protects the T cells from the inactivation signal from PD-L1 expressed by cancer cells or immune regulatory cells. Nivolumab is a PD-1 blocking antibody, which has shown an objective clinical response rate in 30 % of the patients with MM.[3]

The expression of the metabolic enzyme IDO is induced on tumor cells upon exposure to IFN-γ. Activation of IDO inhibits cytotoxic T cells by depleting the microenvironment of amino acids crucial to the function of lymphocytes.[4]

Center for Cancer Immune Therapy (CCIT) has identified spontaneous T cell reactivity against PD-L1 and IDO in the tumor microenvironment and in the peripheral blood of various cancer patients and healthy donors. The IDO reactive CD8+ T cells were cytotoxic and could kill both cancer cells and immune regulatory dendritic cells in vitro. The PD-L1 reactive CD8+ T cells were also cytotoxic and were able to kill cancer cells

and myeloid derived suppressor cells (MDSCs). Thus boosting specific T cells that recognize immune regulatory proteins such as IDO and PD-L1 may directly modulate immune regulation. [5,6,7,8,9,10,11]
Due to the distinct mechanisms of action, the combination of treatment with a monoclonal antibody targeting PD-1 and peptide vaccination with peptides against PD-L1 and IDO will have synergistic effects. Nothing suggests that combining Nivolumab with the experimental peptide vaccine consisting of IDO long and PD-L1 long1 peptides should be more toxic than treatment with Nivolumab alone (see section 12.7).

1.3 Toxicity

IDO

The IDO peptide has been tested in patients with non-small-cell-lung-cancer (NSCLC) (short peptide sequence of 9 amino acids) and MM (long sequence of 21 amino acids). There were minimal toxicity and no grade 3-4 toxicities which could be associated with the IDO vaccine. [12,13] (See IMPD, appendix 5)

PD-L1

PD-L1 peptide is currently being tested at CCIT in a first-in-human phase I clinical trial in patients with multiple myeloma. Generally peptide vaccines are considered to possess very low risk of toxicity.[14] (See IMPD, appendix 5)
Since PD-L1 has a similar expression profile and mode of induction (IFN-γ) nothing suggests that the side effects of a PD-L1 peptide vaccine will differ from IDO vaccines.

Montanide ISA 51

Montanide ISA 51 can cause local discomfort at the insert site but does not usually cause any systemic side effects with the dose used in this trial. (See product resume, appendix 12)

Nivolumab

Grade 3-4 adverse events are seen in around 10-15 % of the patients treated with monotherapy Nivolumab. Half of the side effects involve the gastrointestinal tract, lungs, the endocrine glands, the liver or the skin. The side effects are generally manageable, and the patients are observed closely by an experienced staff at the Department of Oncology. (See product resume, appendix 13)

1.4 Target population

Patients with metastatic malignant melanoma, who according to usual guidelines and clinical evaluation are suited for treatment with Nivolumab.

1.5 Number of participants

The study is designed as an open phase I/II trial. In phase I, 6 patients with MM will be treated. Before phase II can start, all 6 patients must receive the first 4 treatments without any grade 3-4 adverse events, other than those expected with Nivolumab.
If 3 or more patients experience grade 3-4 AE in phase I associated with the vaccination, the trial will be stopped. In phase II, additionally 44 patients will be included.
Three patient cohorts will be included; Cohort A: Anti PD-1/PD-L1 naïve patients (30 patients). Cohort B: Patients with progressive disease ON anti-PD-1 antibody monotherapy (10 patients). Cohort C: Patients with progressive disease during follow up OFF anti-PD-1 after clinical benefit (SD/PR/CR) on anti-PD-1 antibody therapy (10 patients).

1.6 Patient recruitment

The trial is expected to begin inclusion in March 2017. Patients with malignant melanoma will be recruited from the Department of Oncology, Herlev & Gentofte University Hospital, but can also be referred from other centers in Denmark. Patients will receive vaccines for up to 47 weeks and will be followed until progression. The inclusion period is expected to extend over 24 months.

1.7 Treatment plan

Patients included in the trial will be treated with Nivolumab in accordance with the standard regimen, which at the moment involves outpatient IV infusions every second week as long as there is a clinical effect.
The PD-L1/IDO vaccine is given from the start of Nivolumab and every second week for the first 6 vaccines and thereafter every fourth week up 47 weeks. 15 vaccines will be given in total.
At the end of vaccination, patients who are not excluded from the protocol because of progression will continue treatment with Nivolumab in accordance with the usual guidelines.
Patients who receive all vaccines will have follow up appointments after 3 and 6 months in the experimental unit (EFEK) Herlev Hospital in parallel with the standard of care treatment for Nivolumab.

1.8 Toxicity evaluation

Systematic recording of possible adverse events will take place during the course of treatment and follow up in accordance with the Common Terminology Criteria (CTCAE). Recording will take place during patient attendance in the outpatient clinic or the hospital ward at the Department of Oncology, Herlev Hospital.

1.9 Immunological evaluation

Blood samples
In order to monitor the immune response and immune related changes during treatment, project blood samples will be taken before, during and after treatment. Project blood samples will be taken every third month up to 5 years. (See 12.5.1)

Tumor biopsy

At baseline and after the sixth vaccine, tumor biopsy will be done if the tumor is accessible. The objective is to evaluate the tumor immune microenvironment of each patient. Immunohistochemistry, gene expression quantification on different immune genes and whole exome sequencing to assess initial mutational status will be conducted. [15,16] (See 12.5.2)

Delayed type hypersensitivity

Delayed type hypersensitivity (DTH) skin-test and punch biopsy from the DTH area will be done after the sixth vaccination for the evaluation of skin infiltrating lymphocytes (SKILs) reactive to PD-L1 and IDO. (See 12.5.3)

1.10 Clinical response evaluation

Clinical response evaluation will be performed with diagnostic imaging according to RECIST 1.1[17], PERCIST[18] and immune related response criteria (irRC)[19] as well as clinical examination before, during and after treatment. For further details, see the treatment schedule.

Background

2.1 Malignant melanoma

2 Malignant melanoma (MM) is the 5th most commonly diagnosed cancer in Western Europe. Unlike other solid tumors, which mainly affect elderly adults, melanoma also significantly affects young and middle-aged people. Incidence rates have been increasing for the last 15 years of about 3-5 %. In Denmark, 2200 people are diagnosed with malignant melanoma (2014).[20] 5-10 % of these patients have metastases by the time of diagnoses and around 10-15 % develop metastases later in the course of disease.[21] There are around 350-400 new cases of patients with metastatic malignant melanoma per year in Denmark. MM is a very aggressive cancer with a bad prognosis where traditional oncological treatments such as surgery, chemotherapy and radiation therapy have a poor effect, and the 5 year overall survival has hitherto been less than 10 %.[22]

2.2 Standard treatment of malignant melanoma

Huge advances have been made in the treatment of MM in the past 5 years with multiple drugs being approved. In Denmark, 5 treatments are currently approved; Pembrolizumab/Nivolumab, Ipilimumab, Dabrafenib/Trametinib, Temodal, Interleukin-2 (IL-2) + Peginterferon.

Currently, a PD-1 blocking antibody (Pembrolizumab or Nivolumab) is first line treatment and yields a response rate of about 30 % with about 5 % complete response rate and a 3-year overall survival rate of 40 %. [23,24]

Ipilimumab, another immune checkpoint blocking antibody against cytotoxic T lymphocyte associated protein 4 (CTLA-4) is used in the second line and yields a response rate of about 10-15 %, but importantly a fraction of treated patients experience long term stabilization of disease with about 20 % being alive more than 8 years. [25,26]

Combining Nivolumab (PD-1 antibody) and Ipilimumab for the treatment of MM is already EMA approved and is has recently been approved by the Danish authorities as first line treatment to a group of MM patients.[27]

Patients with BRAF mutated melanomas have an additional treatment option after the approval of multiple BRAF and MEK inhibitors. Currently, the combination of Dabrafenib and Trametinib is the treatment of choice, with a response rate of 70 % and significant prolongation of overall survival. Unfortunately, the anti-tumor effect of this treatment is only temporary and eventually all patients progress. [28,29]

Temozolomide is a chemotherapeutical treatment which has an objective response rate (ORR) of 15 % but no general effect on overall survival.[30]

IL-2 and peginterferon are immune stimulating cytokines. Combination of IL-2 and peginterferon is applied for patients under 70 years in a good health condition and has shown an ORR of 15 % and complete response in less than 5 %.[31]

2.1 Immune activation and regulation

In this section, a very brief introduction is given to the adaptive immune system, which is one of the two major divisions of the immune system and very important in the fight against cancer. Cells and molecules of the other division of the immune system, called the innate immune system, also play their important roles in cancer, but the vaccination therapy described in this protocol has its main effects in the adaptive immune system. The adaptive immune system has two branches comprised of the humoral and the cellular branch.

In the humoral branch of the adaptive immune system, antibodies are the major effectors. Antibodies bind to molecules on their targets, whether the targets are bacteria, virus, fungi or malignant cells. Antibodies exert their function by physically blocking surface molecules on the target or by one of several ways mediating specific toxicity to the target. When a surface molecule is blocked by an antibody, the function of the surface molecule can be inhibited. Antibodies can kill targets by cross-linking molecules on the target, by activation of attack-molecules of the complement system to make holes in the target's membrane or by activating other immune cells to kill the target.

In the cellular branch of the adaptive immune system, the foot soldiers are the T cells. Two subgroups of T cells are the cytotoxic T cells (CTLs) and the helper T cells. While the main role of CTLs is to directly kill cells that are virally infected or malignantly transformed, T-helper cells come in many subtypes and have many different roles in the immune system.

CTLs are very effective killer cells, and are probably therefore highly regulated. The most fundamental level of regulation comes from the principle that CTLs can only kill other cells, if the T cell receptor (TCR) of the CTL recognizes a "peptide-fingerprint" on the target cells. Peptides are small pieces of proteins. This fingerprint is a specific peptide epitope bound to a specific major histocompatibility-class I molecule, together making a peptide:MHC-complex. All nucleated cells express these MHC-molecules that display to the immune system a random sample of which proteins are in the cell. If a cell is infected with a virus, viral peptides will be displayed. If a cell has turned malignant, cancer peptides will be displayed.

A T cell has many TCRs, but all TCRs on a T cell are identical. Therefore, a CTL is specific to one peptide-fingerprint. TCRs are made randomly, but whether a specific TCR is expressed on any T cells circulating the body is regulated in the thymus. In the thymus, T cells are positively and negatively selected based on what their TCR recognizes, and how strongly it is recognized. If the TCR binds nothing, the T cell dies of neglect. If the T cells' TCRs bind self-peptides too strongly, the T cell is eliminated.

After the cytotoxic T cell has left the thymus and circulates in the body, another level of regulation is added. This second level of regulation is based on the principle that CTLs must be primed by antigen-presenting cells (APC), often a dendritic cell, to be able to start killing target cells (see Figure 1). APCs eat antigens they meet, chop up the antigens into peptides and present them on MHC-molecules. Priming happens in lymphoid tissue when a CTL meets an APC that has taken up antigen epitopes that the CTL is specific to. The APC delivers a "signal one" to the T cell which is when the APC presents the peptide epitope on a MHC-molecule to the T cell. To be activated, the T cell needs a "signal 2" which it also gets from the dendritic cell because the dendritic cell expresses costimulatory proteins

that activate the T cell. Once a CTL has been primed, it circulates the body and is ready to engage target cells in the effector phase (see Figure 2). In the effector phase, CTLs kill target cells that on the surface have peptide:MHC-complexes that the CTL is specific to.

A third level of regulation is built into the priming and effector phases. Not only does the killing of a target cell depend of a signal 1 and a signal 2 in the priming phase combined with a complementary TCR-peptide:MCH match in the effector phase; what is further needed is the correct balance of so called co-stimulatory and co-inhibitory signals in both phases. In the effector phase one of the very potent inhibitory molecules is programmed death ligand 1 (PD-L1) on the target cell. If a CTL recognizes a peptide:MHC-complex but is inhibited by PD-L1 on the target cell interacting with its receptor PD-1 on the T cell, the T cell is left in an inactive state (see Figure 2A). The negative regulation in the effector phase is an important system to fine-tune the regulation of CTLs, shutting down self-reactive T cells that have slipped through the previous levels of regulation. The balance between stimulatory and inhibitory molecules acts to ensure that the T cells are activated when needed, but prevents T cells from causing damage to normal tissues by inhibiting the T cell response.

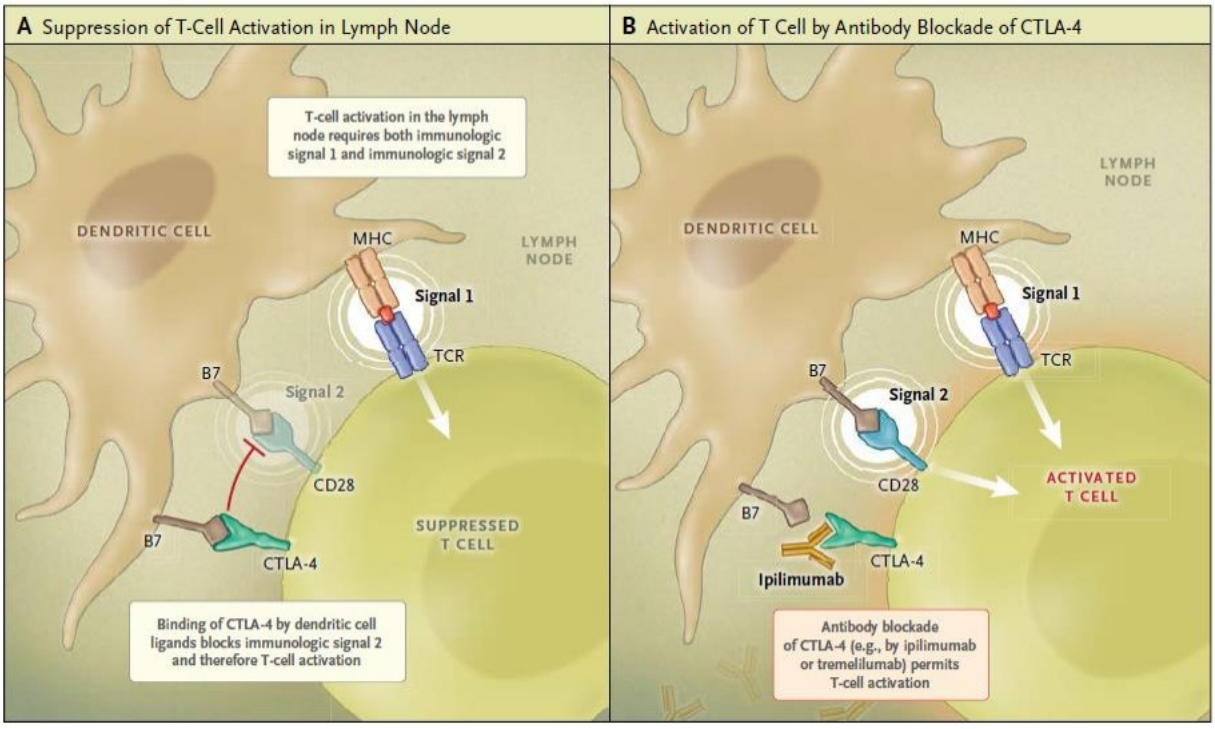


Figure 1: Immune checkpoints and checkpoint inhibitors, the priming phase. In the priming phase, an antigen presenting cell activates antigen-specific T cells by stimulating via both the MHC-TCR-pathway (signal one) and the co-stimulatory B7-CD28-pathway (signal two). In the priming phase, the immune responses can be inhibited if the T cells have upregulated the checkpoint molecule CTLA-4. CTLA-4 can be blocked by the checkpoint-inhibiting antibody Ipilimumab. (Ribas, NEJM 2015[32])

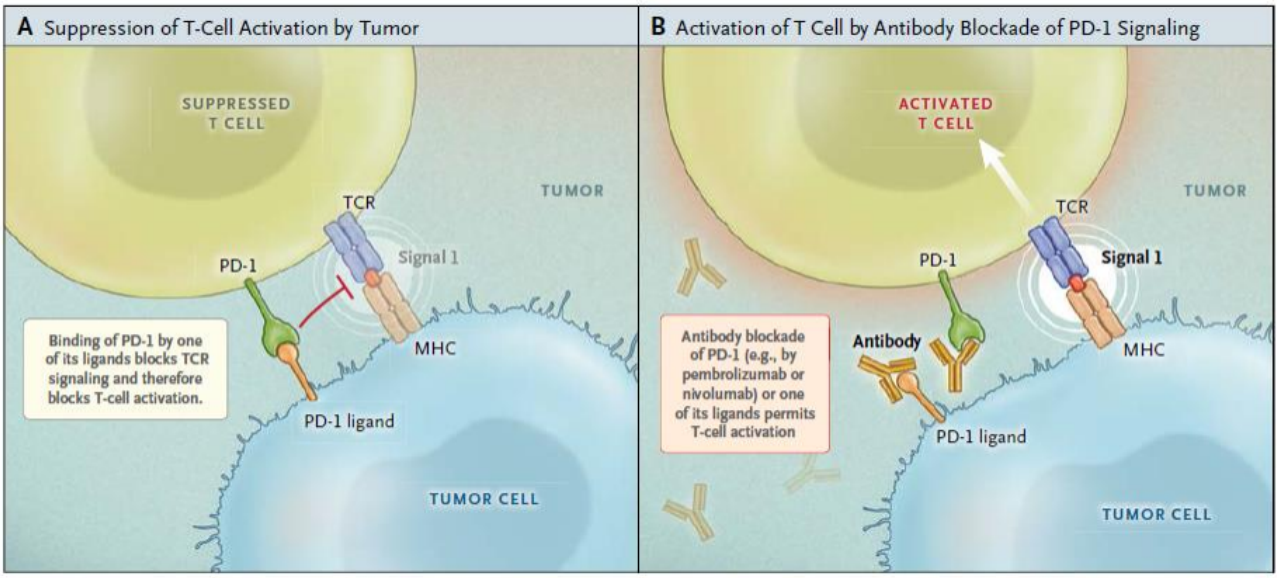


Figure 2: Immune checkpoints and checkpoint inhibitors, the effector phase. In the effector phase, antigen-specific T cells recognize target cells presenting the target peptide-MHC-molecule. In the effector phase, immune responses can be inhibited by the checkpoint molecules PD-1/PD-L1. Several antibodies blocking PD-1 or PD-L1 have been marked. (Ribas, NEJM 2015[32])

2.2 Cancer cells induce local tolerance

The unregulated growth of cancer cells is only possible when the immune system does not launch an effective antineoplastic response against the cancer. Impairment of the immune system is thus a hallmark of cancer.[33] Strikingly, the insufficient immunological activity in cancer patients seems not to be a general impairment of the immune system. Most cancer patients do not suffer from frequent infections, except when their immune system is very affected by treatment or when the cancer has reached a late state with profound impact on the body. Thus, the impairment of the immune system must be somewhat specific to the cancer cells and the local microenvironment around the tumor. This discrepancy between a near normal overall immune function and profound immunosuppression locally can be explained by the increase in immune regulatory molecules and immune regulatory cells in and around the tumor.

Cancer cells highjack the system of checks and balances of T cell activation by overexpressing inhibitory molecules such as PD-L1 and regulatory enzymes such as IDO. Furthermore, immunosuppressive regulatory cells that also express inhibitory molecules like PD-L1 and IDO are recruited to the tumor. Combined this causes a very immunosuppressive local environment, skewing the normal balance between stimulating and inhibitory signals in the direction of immunosuppression in and around the tumor. The co-inhibitory molecules CTLA-4, PD-L1 and PD-1 are checkpoints in the immune system. One of the major pillars of the recent impressive advance of the field of immune-oncology is the introduction of the so called immune checkpoint blocking monoclonal antibodies. Antibodies blocking the action of CTLA-4 in the priming phase or PD-1/PD-L1 in the effector phase block out these negative signals in the interaction of T cells with APC or target cells, respectively (see Figures 1B and 2B). Blocking the negative signals in the balance "releases the break" on T cells, allowing them to kill malignant cells.

All in all, cancer cells have many mechanisms whereby they escape immune attack. Two of the mechanisms, i.e. the immune inhibitory pathway PD-1/PD-L1 and the metabolic enzymes will be described further in section 2.3 and 2.4.

2.3 IDO and its potential as a vaccination antigen

2.3.1 IDO and its function

IDO is an immunoregulatory enzyme that has crucial functions in immune suppression and homeostasis, and is implicated in suppressing T cell immunity in cancer.[34] IDO is a major inhibitor of the effector phase of the immune response, and suppresses effector T cells directly by degradation of the essential amino acid tryptophan. The biological effect of IDO is mediated in part through local depletion of tryptophan, but also via immune-modulatory tryptophan metabolites.[4] Regulation of tryptophan metabolism by IDO in dendritic cells (DC) is a highly adaptable modulator of immunity: IDO+DC are capable of inducing immune suppression and anergy in antigen-specific T cells in the lymph node draining the injection site. Effector T cells starved of tryptophan are unable to proliferate and go into G1 cell cycle arrest via activation general control nonderepressible 2 kinase (GCN2) pathway. GCN2 is a stress-response kinase that is activated by elevations in uncharged transfer RNA (tRNA), as would occur if the T cells were deprived of tryptophan. Finally, IDO enhances local regulatory T cell mediated immune suppression: constitutive IDO expression in DC provides T cells with regulatory properties that block T cell responses to antigenic stimulation.[35,36]

IDO expression is widely dysregulated in cancer patients. IDO contributes in critical manner to inhibit or terminate inflammation and is highly over-expressed in cancer. In cancer patients, elevation of IDO expression occurs in a subset of plasmacytoid DC in tumor-draining lymph nodes.[37] In addition, IDO may be expressed within the tumor microenvironment, including tumor stromal cells, where it inhibits the effector phase of immune responses.[38] Activation of IDO in either tumor cells or nodal-regulatory DC each appears to be sufficient to facilitate immune escape of tumors. In this regard, it has been described that expression of IDO in tumor cells is associated with an impaired prognosis. Tumor cells transfected with IDO become resistant to immune eradication in a murine model where a fully protective immune response had been established by immunization.[39] IDO expressing CD19+ plasmacytoid DC isolated from tumor-draining LN mediates profound immune suppression and T cell anergy in vivo, whereas plasmacytoid DC from normal LNs and spleen do not express IDO.

Conclusively, IDO is a critical cellular factor contributing to the suppression of anti-cancer immunity and as such, IDO is most likely a crucial mechanism in cancer progression. Hence, IDO has become a very attractive target for the design of new anticancer drugs.

2.3.2 Preclinical data on IDO

Demonstration that T cells recognize IDO-derived peptides

A shorter peptide, IO101, which is an HLA-A2-restricted, IDO derived CD8+ T cell epitope ($IDO_{199-207}$; ALLEIASCL), was initially identified. IO101 contains only MHC class I-restricted CD8+ T cell epitope that is

contained within the longer IO102 (also named IDO long) sequence (which contains both CD8+ T cell epitopes as well as CD4+ T cell epitopes; $IDO_{194-214}$ DTLLKALLEIASCLEKALQVF).
Spontaneous T cell reactivity towards both of these IDO-derived peptides have been detected in cancer patients bearing different tumor types (i.e. melanoma), which was measured by flow cytometry using HLA:peptide tetramers, as well as in ELISPOT assays after in vitro stimulation but also in direct ex vivo assays. In addition, it was demonstrated that T cell reactivity against both IO101 and IO102 to a lesser extent exists in healthy individuals. Interestingly, in all patients, we detected both CD4 and CD8 T cell responses against IDO, suggesting that class I- and II restricted IDO responses co-develop. [40,41,42]

Demonstration that IDO-specific T cells can kill tumor cells

IDO-reactive CD8+ T cells are peptide specific, cytotoxic effector cells: IDO specific T cells can effectively lyse IDO+ cancer cell lines of different origins, such as melanoma. Interestingly, circulating IDO specific T cells can be isolated from not only cancer patients but also healthy individuals, and these T cells exhibit similar cytotoxic functions against IDO-expressing malignant cells. Furthermore, the addition of known IDO-inducers like IFN-gamma cause the expansion of IDO specific T cells among PBMCs, thus the level of IDO expression in PBMCs positively correlates with the frequency of IDO-specific T cells. Finally, the relevance of IDO-specific CD4+ T cells in cancer was further emphasized by the findings that IDO specific T cells reacted towards dendritic cells (DC) pulsed with IDO+ tumor lysates. [40,41,42]

Demonstration that IDO-specific T cells enhance activities of other effector T cells

IDO specific cytotoxic T cells can boost activation of other T cells towards viral and tumor-associated antigens. This “supportive” effect on T cell immunity by IDO specific T cells was mediated in several direct and indirect manners. First of all, IDO specific T cells were capable of killing IDO-expressing regulatory cells thereby directly targeting the IDO-dependent counter-regulatory pathway. Thus, when IDO-specific T cells were activated, the IDO activity decreased, the level of tryptophan was elevated, and T cell activities were enhanced. Furthermore, IDO specific T cells enhanced overall production of pro-inflammatory cytokines, like TNF-α and IL-6, but a decrease in IL-10. Moreover, the metabolites of tryptophan are known to be toxic to CD8+ T cells and CD4 Th1 cells, but not Th2 cells. Hence, increased IDO activity appears to favor the polarization of helper T cells toward a Th2 phenotype. Conversely, activation of IDO-specific cytotoxic T cells may drive T-helper polarization in the Th1-direction, which is considered beneficial for protective immunity in cancer. Lastly, IDO produces kynurenine, which may effectively hamper the immune response by binding the aryl hydrocarbon receptor favoring the local formation of Tregs. Hence, targeting IDO-positive T cells should decrease the number of Tregs. Indeed the frequency of Tregs decreased when IDO-specific T cells were activated. [40,41,42]

Conclusion on preclinical data on IDO peptide

In conclusion, IDO-specific T cells are able to directly recognize and kill IDO+ cancer cells and IDO expressing regulatory T cells. IDO specific T cells are naturally present in cancer patients and to a lesser extent in healthy donors. IDO will likely serve as a new and widely applicable target for immunotherapeutic strategies.

Vaccination with an IDO peptide has been tested in humans, see section 2.10.1 and 2.10.2

2.4 PD-L1 and its potential as a vaccination antigen

2.4.1 PD-1/PD-L1 axis

The PD-1 receptor was discovered in 1992, as a protein that was upregulated during the apoptosis of lymphocytes, hence the name.[43] The main action of PD-1 is negative regulation of T cell activity. PD-1 is expressed on monocytes, dendritic cells, T cells, B cells and natural killer (NK) cells. PD-1 is upregulated on T cells when they are activated, but persistent expression of PD-1 is a marker of T cell exhaustion, an unresponsive state of inactivity.[44]

The PD-1 ligand PD-L1 (B7-H1) was discovered in 1999 and is a 290-amino acid transmembrane protein encoded by the CD274 gene.[45,46] The extracellular portion of PD-L1 comprises IgV- and IgC-like domains, while the intracellular part comprises a 30-amino acid tail.[45] The expression of PD-L1 in normal non-inflamed tissues has reported either not expressed[47–49] or only expressed in low amounts[50–53] apart from on macrophages which are clearly PD-L1 positive.[47] Whether PD-L1 expression can be found when investigating normal tissues, can be boiled down to the sensitivity of the technique. PD-L1 expression is induced in normal cells if they are in an inflammatory environment by stimulation with the inflammatory cytokines such as interferon-γ (IFN-γ).[47–53]

When PD-L1 interacts with PD-1 on a T cell, this inhibits the activation of the T cell via interfering with the signaling from the TCR.[54,55] Furthermore, via several other mechanisms, PD-1 stimulation inhibits proliferation, differentiation and cytotoxic functioning of the T cell as well as diminishes its release of inflammatory cytokines and leads to poor contact with dendritic cells.[55] PD-L1 plays a critical role in the conversion of naïve T cells to regulatory T cells (Tregs).[56] Signaling into the cancer cell, PD-L1 acts in a manner to reduce apoptosis.[57]

When an activated CTL commences the killing process of a target cell, it excretes the cytokine IFN-γ and upregulates its own expression of PD-1 (Figure 3). This has major implications in the local microenvironment. IFN-γ upregulates both MCH-class I molecules and PD-L1 molecules on the cells near the CTL. More MCH-class I molecules mean better presentation of peptides from the cells in the neighborhood, which improves the chance that CTLs will find suited target cells. But the upregulation of PD-L1 combined with the activation induced PD-1 on the T cell itself pulls the balance in the direction of shutting down the immune response. This is a normal limitation of T cell activity.

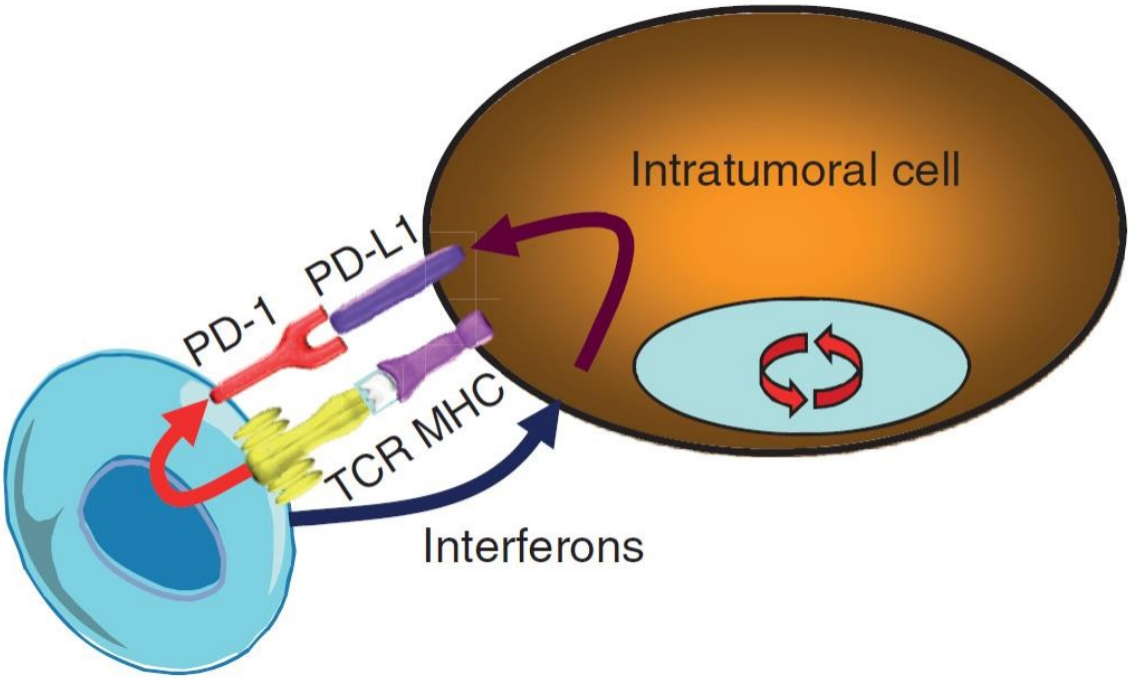


Figure 3: Interferon upregulates PD-L1. When CTLs recognize their specific tumor antigen, both PD-1 on the T cell and PD-L1 on the tumor cell are expressed due to IFN-*γ* secreted by the CTLs. MHC-expression is also increased, not shown in this figure. (Ribas, Cancer Discovery 2015[58])

2.4.2 Preclinical data on PD-L1

Demonstration that T cells recognize PD-L1-derived peptides

The PD-L1 long1 (PD-L1$_{9-27}$) peptide contains both CD8+ T cell epitopes as well as CD4+ T cell epitopes. Spontaneous T cell reactivity towards PD-L1 long1 peptide have been detected in both healthy individuals and cancer patients including MM, which was measured by flow cytometry using HLA:peptide tetramers, as well as in ELISPOT assays after in vitro stimulation but also in direct ex vivo assays. The T cell reactivity was higher in cancer patients, and both CD4 and CD8 T cell responses against PD-L1 long 1 was detected. These PD-L1 specific CD8+ T cells release IFN- γ and TNF-α. Notably, a few individuals in whom we were able to measure specific T cell responses directly ex vivo had a relatively high number of PD-L1 specific T cells.[9]

This is especially noteworthy as detection of conventional tumor-associated antigen specific T cells, with a few exceptions, are not possible to detect in PBMC from cancer patients directly ex vivo without prior in vitro peptide stimulation.[59]

Demonstration that PD-L1 long1 T cells can kill tumor cells

Further analyses demonstrated that the PD-L1 specific T cells in PBMCs were cytolytic effector cells using Granzyme B ELISPOT assay. In addition, PD-L1 specific T cell cultures were generated by re-stimulating PBMCs with the PD-L1 peptide in vitro and showed that the subsequent T cell lines were PD-L1 specific.

Furthermore, we found that the PD-L1 specific CD8+ T cells were cytolytic effector cells that can recognize and kill PD-L1-expressing melanoma cells.[10]

Demonstration that PD-L1 specific T cells directly and indirectly augment other T cell responses

The PD-L1/PD-1 pathway is important for the regulation of both viral and anticancer CTL responses. PD-L1 specific CTLs were therefore used to influence antiviral immunity. The addition of PD-L1 specific CTLs in culture 1 week after virus epitope stimulation resulted in an increase in the number of virus-specific CD8+ T cells. An increase was also found in cultures co-stimulated with the PD-L1 peptide epitope compared to cultures that were co-stimulated with an irrelevant epitope from HIV-1. Hence, PD-L1 specific CTLs may efficiently augment the effector phase of the immune response by suppressing PD-L1 expressing regulatory cells that restrain PD-1-expressing effector T cells.[60,61]

PD-L1 long1 enhances the immune response towards a DC vaccine

A DC vaccine was recently tested in patients with stage IV melanoma. In the study, patients were vaccinated with allogeneic DCs transfected with mRNA encoding the tumor-associated antigen p53, survivin and telomerase (called DCvacc in the following). However, clinical benefit was limited with low reactivity towards DCvacc in patient PBMCs.

To investigate if PD-L1 long1 could enhance the reactivity towards DCvacc, in vitro co-stimulation experiments where PBMCs from patients with malignant melanoma were stimulated with DCvacc and PD-L1 long1, or DCvacc and an irrelevant control peptide.

The results show a significant increase in the T cell reactivity against DCvacc when patient PBMCs were co-stimulated with PD-L1-derived peptides. Reactivity of $CD4^+$ T cells increased the most, but $CD8^+$ T cell reactivity was also significantly boosted by co-stimulation.

This suggests that the addition of PD-L1 epitopes to a cancer vaccine could strengthen immune responses against the vaccine in vivo.

Conclusion on preclinical data on the PD-L1 peptide

PD-L1 specific T cells are naturally occurring in both cancer patients and to a lesser extent in healthy donors. PD-L1 long1 can induce broad CD4 and CD8 T cell drive immune responses towards PD-L1 expressing cells including cancer cells and cells of the immune system. Adding PD-L1 specific T cells to virus specific T cells, or as co-stimulation with DC-vaccine results in enhanced expansion of the virus or DC-vaccine specific T cells. Vaccination with the PD-L1 peptide is currently being tested in a first-in-man study in patients with multiple myeloma, see section 2.10.3.

2.5 Mechanism of Action – vaccination therapy

All nucleated cells are characterized by a "peptide-fingerprint" (MHC:peptide complex) on the cell surface, which to the outside exposes a mirror of the protein content in the cell. Thus, cancer cells as well as immunosuppressive immune cells that overexpress PD-L1 and IDO will exhibit PD-L1 peptide:MHC and IDO peptide:MHC complexes on the cell surface and can be recognized by T cells. [62,63]

The principle of vaccination is to administer the target (the antigen) recognized by the immune system together with an immune enhancer, the adjuvant. The antigen can be composed in many different forms. Relevant examples in cancer vaccination are whole tumor cell vaccinations in which killed tumor cells are used as the source of antigen. Obviously, such an antigenic source comprises a huge number of proteins as potential antigens, many of which will in fact also be present in normal cells.

Administered in combination with a relevant adjuvant, cellular proteins derived from tumor cells will be taken up by antigen presenting cells (APC). APC will process the proteins, i.e. cut the proteins into peptides that are capable of binding to both class I and class II MHC molecules. MHC:peptide complexes expressed on the surface of APC may elicit T cell responses specific for the individual peptides – and these T cells thus have the capacity to recognize cancer cells, which express an identical MHC:peptide molecule on the cell surface. At the other extreme, an antigen used for vaccination may simply be a peptide of 9-amino acids derived from a protein expressed by cancer cells. This form of antigen negates the need for antigen processing step by APC as the peptide is already in a form ready to be presented by the APC in the context of MHC. Such peptide antigen may activate T cells capable of recognizing the identical peptide sequence expressed on the surface of cancer cells in the context of MHC class I molecules. Both approaches have advantages and shortcomings.

Long peptides of approximately 15 to 20 amino acids have been used in therapeutic vaccination studies for more than a decade and can be viewed as an attempt to develop a strictly defined yet still broadly applicable target antigen. A main advantage of the long peptide approach compared with the conventional short peptides is that such peptides can encompass epitopes binding with both MHC class I and II, i.e. both inducing CD4 T helper responses as well as CD8 cytotoxic T cell responses. Importantly, peptide binding to MHC class II is rather promiscuous, and any given peptide may be capable of binding to several different MHC class II molecules.

Indeed, both the IDO long peptide and the PD-L1 long1 peptide can be recognized by both CD4 and CD8 T cells.

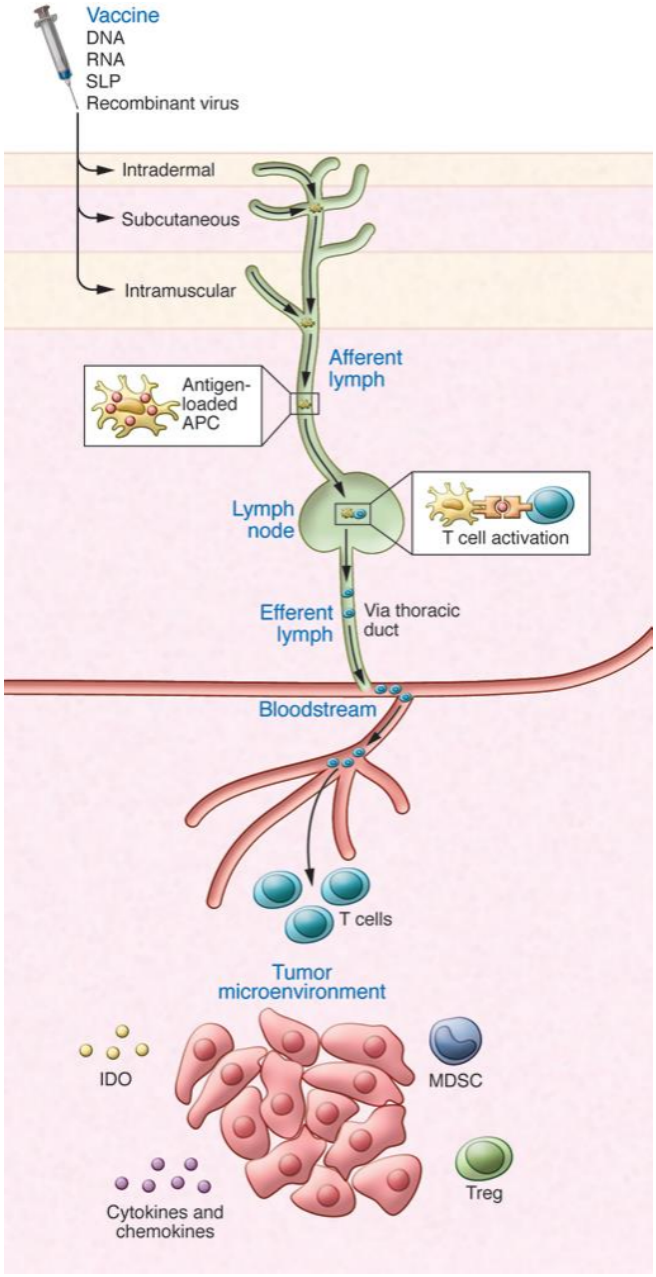


Figure 5: Principle of therapeutic cancer vaccination. APCs take up antigen at the injection site and migrate to a lymph node. During migration to the lymph node they mature to efficient antigen presenters, this is improved if a strong adjuvant provides a good "danger"-signal. In the lymph node, APCs meet T cells and prime them. When T cells are primed, they leave the lymph node to circulate the body patrolling for targets. APC: antigen-presenting cell. DNA: Deoxyribonucleic Acid. IDO: Indoleamine 2,3-deoxydase. MDSC: myeloid derived suppressor cell. SLP: synthetic long peptide. RNA: Ribonucleic Acid. Treg: regulatory T cell. (Melief, JCI 2015) [64]

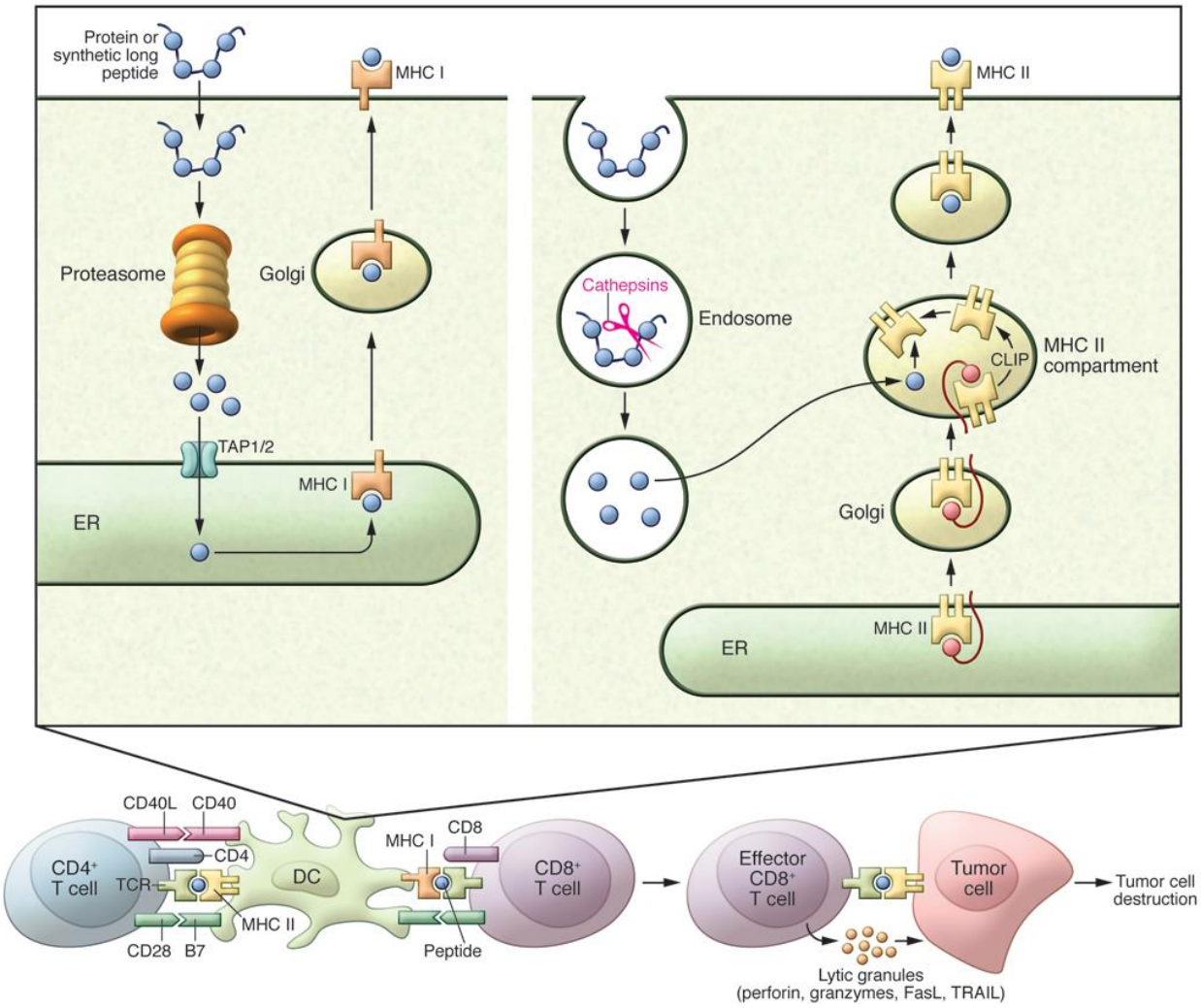


Figure 6: The processing of long peptides in APCs allows stimulation of both CTL and T-helper cells. In the right side of the figure, a long peptide is taken up via endocytosis, cut into pieces and presented on MHC-class II molecules. This allows it to be presented to CD4-positive T-helper cells. In the left side of the figure, a long peptide enters the cytosol. The jury is out as to how it enters the cytosol, but we know it happens. From the cytosol, it is processed like a normal protein from the cell. This normal way is by degradation in the proteasome, transportation into the ER and in the ER loading onto MHC-class I molecules. When a peptide is bound to a MHC-class I molecule, it is presented to CD8-positive CTLs. In this manner, a long peptide is not restricted to either class I or class II MHC-molecules. DC: dendritic cell. ER: endoplasmatic reticulum. TAP1/2: Transporter associated with Antigen Processing1/2. (Melief, JCI 2015[65])

PD-L1 and IDO are normal proteins which are overexpressed by cancer cells including melanoma cells. In the case of this vaccine against PD-L1 and IDO, we have found PD-L1 and IDO specific T cells in both healthy donors and cancer patients. Interestingly, we have previously described that patients harboring a spontaneous T cell response towards IDO prior to vaccination performed clinically better when vaccinated with an IDO peptide[13].

Once PD-L1 or IDO expressing cancer cells are attacked by T cells recognizing PD-L1 or IDO, remnants of the killed cancer cells will be taken up by APCs. The APCs will process antigens from the cancer cells and

present their cancerous neoantigens to other T cells. This process is called epitope spreading and is thought to be one of the underlying causes to the success of cancer immune therapy.
Vaccine activated PD-L1 or IDO specific T cells will engage at the tumor micro-environment and attack cancer cells. However, PD-L1 or IDO specific T cells do not attack normal cells in non-cancerous tissues, since the expression level of PD-L1 and IDO is much lower. It requires a high expression of PD-L1 and IDO in a cell to produce a high enough amount of PD-L1-peptide:MHC-complexes on the surface to overcome the threshold of being killed by PD-L1 or IDO specific T cells. The affinity of T cell receptor to MHC:peptide complexes are 100-1000 fold lower than that between antibody and antigen. Consequently, many PD-L1-peptide:MHC-complexes and IDO-peptide:MHC-complexes have to be on the cell surface for the activation signal to be strong enough. Furthermore, the activation of PD-L1 or IDO -specific T cells is a slow process. Therefore, only by vaccinating several times, the amount of anti-PD-L1 or anti-IDO T cells will be sufficient. Finally, it is well described that "danger signals" are vital for the effector phase of an immune response. Thus, T cells preferentially engage in areas of inflammation with dying cells and other sources of "danger signals"[66]. These signals are present in tumors but not in normal, healthy tissue. This further explains the general lack of toxicity in anti-cancer vaccinations utilizing self-antigens.

2.6 PD-1 and PD-L1 blocking antibodies

Multiple studies of anti-PD-1 and anti-PD-L1 blockade report the subsequent restoration of T cell effector function and proliferation as well as increased infiltration of tumors by cytotoxic T lymphocytes (CTLs). This alters the balance between cytotoxic cells and immunosuppressive cells and ultimately results in the death of tumor cells. The blockade of either PD-1 or PD-L1 by monoclonal antibodies has led to outstanding clinical responses, and the Food and Drug Administration (FDA) recently approved the anti-PD-1 antibodies Pembrolizumab (Keytruda) and Nivolumab (Opdivo) in September and December of 2014, respectively. Blocking the PD-1 pathway shows great clinical promise, and there is high commercial interest and intense competition among drug companies to develop agents that target PD-1 or PD-L1.

2.7 Adjuvant

As described in section 2.6, a peptide antigen is administered with an immune enhancer, an adjuvant. The adjuvant increases the uptake of the antigen by APCs, and mediates so-called "danger signals" to the APC. When an APC takes up and presents antigen in the context of a danger signal, it will deliver co-stimulatory signals to T cells, which activates T cells that recognize the antigen.
The adjuvant to be used is Montanide ISA-51. Montanide is an incomplete Freund's adjuvant (IFA) which forms water in oil emulsion when mixed with an aqueous phase. It is commercially available from Seppic S.A, France, which maintains a Drug Master File. Montanide has been used in cancer vaccines in other research projects in more than 5000 patients corresponding to more than 50.000 injections subcutaneously or intramuscularly. IFA is used with success in combination with long peptides, where clinically relevant responses can be produced with IFA. For instance in a vaccine with a long HPV-16 peptide against vulva cancer.[67,68,69]

Montanide has a well-documented safety profile. Frequently reported side effects after injection are flu-like symptoms (headache, chills, fever and nausea) while most common local reactions are granuloma, local pain, inflammation or erythema. These reactions are generally classified as mild to moderate and they are typically transient in nature.

2.8 Rationale behind the peptide vaccine with IDO & PD-L1

Cancer cells and other immune regulatory cells, such as tumor-associated dendritic cells (TADC) and MDSC's express inhibitory surface molecules (e.g., PD-L1) and metabolic enzymes (e.g. IDO), which limits the anti-tumor activity of specific anti-tumor T cells in the tumor microenvironment. PD-L1 specific or IDO specific T cells recognize MHC-bound PD-L1 or IDO peptides, and are able to eliminate PD-L1 expressing or IDO expressing immunoregulatory cells and cancer cells. Thus, activation of PD-L1 or IDO specific T cells through vaccination with the PD-L1 and IDO peptides will boost a natural killing of cancer cells and counteract immunoregulatory mechanisms in the tumor microenvironment. In addition, the vaccine should attract specific pro-inflammatory helper T cells to the tumor microenvironment. Thus, IDO or PD-L1 specific T cells may both directly support anti-cancer immunity by killing target cells as well as indirectly by releasing pro-inflammatory cytokines in the microenvironment to boost additional anti-cancer immunity (Figure 7).

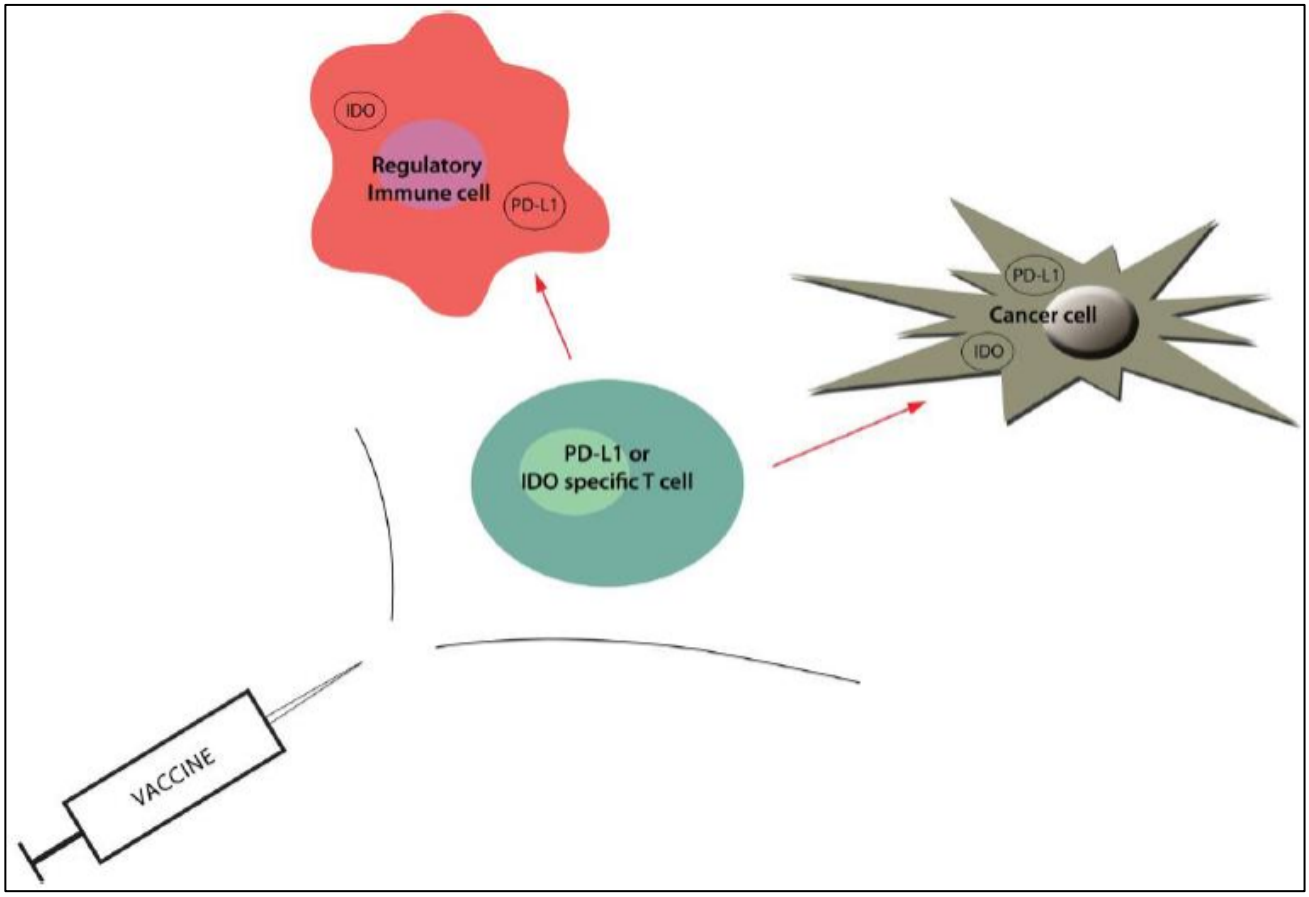


Fig. 7: The peptide vaccine consisting of PD-L1 long1 and IDO long peptide boosts the natural immunity mediated by PD-L1 and IDO specific T. These can attack and kill regulatory immune cells and cancer cells as well as support additional anti-cancer immunity by the release of helper cytokines.

2.9 Rationale behind the combined treatment strategies

As described earlier, antibodies blocking PD-1 (Nivolumab) have shown an OR in 30 % of MM patients, but by combining this treatment with a peptide vaccine containing IDO and PD-L1, we aim to antagonize additional immunosuppressive pathways and thereby boost the effect of the PD-1 blocking antibody, Nivolumab. The hypothesis is that the PD-L1/IDO peptide vaccine and Nivolumab will complement each other, since their mechanisms of action differ.

Effective peptide vaccination results in a slowly increasing number of cytotoxic T cells specific to the antigen over the course of multiple repeated vaccinations. In the case of a PD-L1/IDO peptide vaccine, PD-L1 specific and IDO specific cytotoxic T cells will target PD-L1 and IDO expressing cells in the tumor. When cytotoxic T cells engage target cells, the T cells secrete INF-γ to the surroundings and upregulate PD-1 on their surface. When the surrounding cells in the tumor microenvironment are exposed to INF-γ, they further upregulate PD-L1 and IDO. In this manner, the action of PD-L1- and IDO-specific T cells will be self-limiting. Combining peptide vaccination against PD-L1 and IDO with antibodies against PD-L1 will prolong the effect of PD-L1 specific and IDO specific T cells, and thus increase the effect of the vaccination (Figure 8).

Trials with vaccination with IDO peptides have been conducted as monotherapy and in combination with the anti-CTLA-4 checkpoint blocking antibody Ipilimumab and likewise a trial with vaccination with PD-L1 long1 as monotherapy is undergoing. Like all previous peptide vaccination studies, toxicity has been very limited. When combining IDO long peptide vaccination with Ipilimumab, no increase in toxicity was seen compared with what would be expected with Ipilimumab alone.

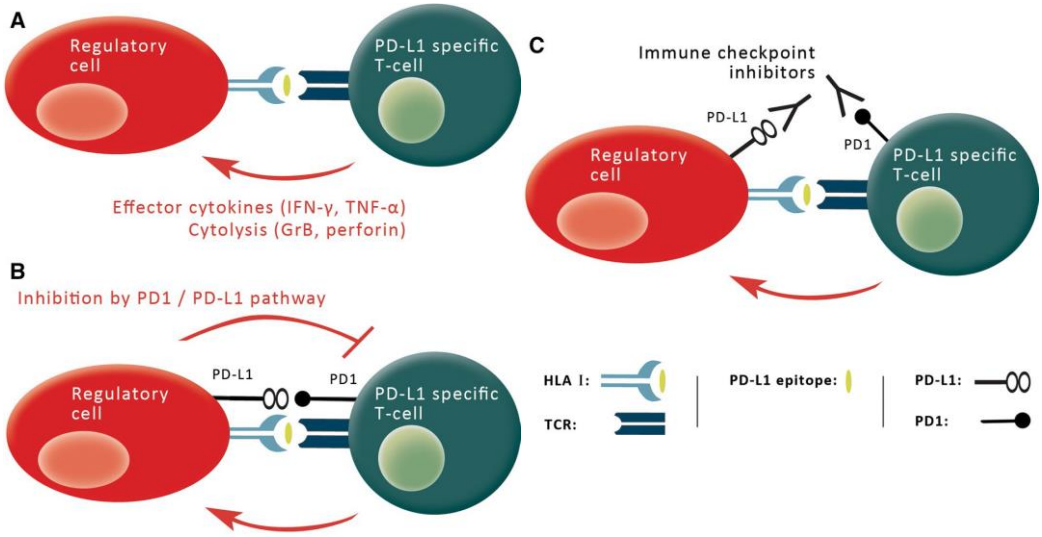


Fig. 8: The self-limiting effects of peptide vaccination-stimulated cytotoxic T cells can be mitigated by concomitant treatment with checkpoint blocking antibodies. A: Cytotoxic T cells recognize target cells via peptide-MHC-complex and secrete effector cytokines. B: Activated T cells upregulate PD-1, and via INF-γ upregulate PD-L1 on cells around them. PD-L1 specific T cells thus have a self-limiting activity. C: Concomitant treatment with immune checkpoint blocking antibodies will act in synergy to prolong the effect of vaccination therapy.

2.10 CCIT's experience with IDO and PD-L1 peptide vaccines

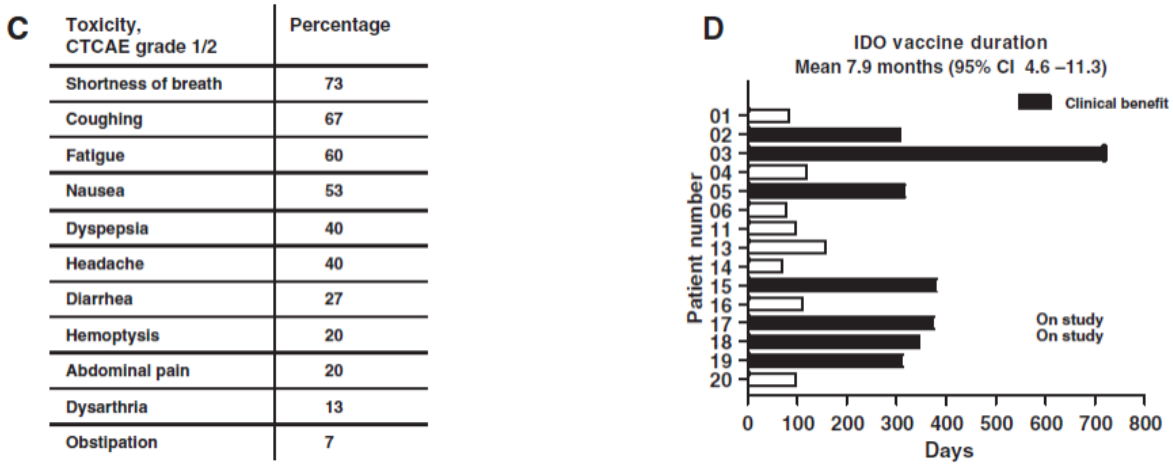
2.10.1 NSCLC and IDO peptide vaccine

A peptide vaccine containing IDO has already been tested in humans. To evaluate the efficiency and safety of IDO-based vaccinations, CCIT conducted a phase I first-in-man clinical vaccination trial. The study comprised 15 patients with advanced non-small-cell-lung-cancer that were vaccinated with an IDO-derived short 9-amino acid peptide in Montanide adjuvant. The vaccine was very tolerated with no toxicities above grade 2.

In the study, the median overall survival (OS) was > 2 years, which was higher than expected for this patient group. In 1 patient with liver metastasis an objective response (PR) was observed. The patient had continuous tumor regression on vaccine treatment for 1 year before qualifying as a partial response. He and one other patient are still enrolled in the trial on the 5th year without signs of progression.

The vaccine comprised an MHC-A2-restricted epitope from IDO. Hence, prior to inclusion in the study, MHC tissue typing was performed. Therefore, it was possible to compare the OS of the MHC-A2+ patients that were vaccinated with the patients that were otherwise eligible for the study but were excluded, since they did not express MHC-A2. The MHC-A2+ patients that were vaccinated had a median survival of 25.9 months demonstrating significantly longer OS compared to the MHC-A2- patients who had a median OS of 7.7 months. Importantly, is was recently described in a large study that expression of MHC-A2 is an unfavorable prognostic factor in stage I NSCLC patients, which underlines the potential importance of the significantly longer OS observed in vaccinated MHC-A2 patients compared to unvaccinated MHC-A2 negative NSCLC patients, though these data need confirmation in large clinical trials.[70]

Immune monitoring revealed that IDO specific T cells were detectable in all patients, thus not only in patients that seem to benefit clinically. In 2 SD patients an IDO-specific T cell response was detected during 1 year of treatment, suggesting sustained long-term IDO reactivity.[13]



C

Toxicity, CTCAE grade 1/2	Percentage
Shortness of breath	73
Coughing	67
Fatigue	60
Nausea	53
Dyspepsia	40
Headache	40
Diarrhea	27
Hemoptysis	20
Abdominal pain	20
Dysarthria	13
Obstipation	7

Figure 9: Toxicity and duration of treatment in 15 patients with metastatic lung cancer vaccinated with a short IDO-peptide. Of note is the absence of grade ≥3 toxicities. Duration of treatment was at the time of publication in 2014. Two patients (numbers 17 and 18, Figure D) still receive vaccinations at present time - 4.5 years after initiation. (Iversen, Clin Cancer Res 2014[13])

2.10.2 MM and IDO peptide vaccine in combination with Ipilimumab

A first-in-man study with IDO long (21-amino acid peptide) was performed in 10 melanoma patients in combination with the standard treatment at that time which was the anti-CTLA4 checkpoint blocking antibody Ipilimumab. The primary objective of the trial was to assess safety and tolerability of the combination treatment. Each patient was vaccinated a total of 7 times. The first 4 vaccines were given weekly and the subsequent 3 vaccines were administered biweekly. Ipilimumab was administered IV at a dose of 3 mg/kg every 3 weeks for a total of 4 treatments. Vaccine related toxicities were below grade 3 as in the previous IDO vaccine study, but as expected, patients experienced toxicity to Ipilimumab. One patient developed presumed Ipilimumab-induced colitis, which initially responded to corticosteroids, but later relapsed while the patient due to unfortunate circumstances was admitted to a local hospital where the patient died after receiving suboptimal therapy. Colitis is a known side effect to Ipilimumab which requires intensive management. Vaccine-specific responses were demonstrated by ELISPOT in 3 of 10 patients.[12]

2.10.3 Multiple myeloma and PD-L1 peptide vaccine after high dose chemotherapy

The PD-L1 peptide is currently being tested in patients with multiple myeloma in a first in man phase I study (EudraCT number 2016-000990-19). There is not yet data on toxicity.
In exploratory MOA murine studies, the human sequence as expected does not induce activation of PD-L1 specific T cells in mice, whereas, vaccination with the murine sequence induces strong responses. No toxicity was found in mice vaccinated with either human PD-L1 long1 & murine PD-L1 long1. Therapeutic peptide vaccines lack a suitable model for safety, see section 2.11.

Vaccination with PD-L1 peptide will resemble IDO vaccination in several ways:

- It is also given with the adjuvant Montanide
- The target protein is also an immunosuppressive self-molecule, which is widely expressed on cancer cells, antigen-presenting cells, and immunosuppressive cells
- IDO is also broadly induced on non-immune cells when their tissue is inflamed e.g. by IFN-γ

Nothing suggests that vaccination against PD-L1 should be more toxic than vaccination against IDO.

2.11 Therapeutic peptide vaccines lack a suitable animal model for safety and dose testing

Therapeutic peptide cancer vaccines are a group of biopharmaceuticals that lack a useful non-human model for nonclinical safety evaluation according to the ICH S6(R1):

1. To identify initial safe dose and subsequent dose escalation schemes in humans
2. To identify potential target organs for toxicity and for the study of whether such toxicity is reversible; and
3. To identify safety parameters for clinical monitoring

According to the guideline it is recommended that safety evaluation is performed in a relevant species where the test material is pharmacologically active alternatively in transgenic animals or by use of homologous therapeutics.

To create an animal model with completely transgenic TCR is not possible. Furthermore, the amino acid sequences of PD-L1, the target molecule in question, differ between humans and other animals, making the assessment of potential autoimmunity impossible using an animal model. Accordingly, it is not feasible to use a transgenic animal model to reproduce the pharmacological activity and toxicity that occurs in the human body because of the administration of peptide vaccines.

The numerous limitations on standard preclinical animal studies have been described in a consensus paper by a collaboration of international societies for cancer immune therapy.[71] This consensus paper underscores that "Even if mice were generated that expressed the appropriate HLA type and the human antigen sequences, such models might not adequately predict safety or autoimmune effects based on the diversity of the other components of antigen processing machinery involved." A clinical development paradigm for cancer vaccines has been proposed by a group of more than 50 experts from academia, regulatory bodies and the biotech/pharmaceutical industry from Europe and the US FDA[72]. In this development paradigm, it is proposed to "…adopt a flexible approach, performing studies likely to be informative, while avoiding tests that contribute little valuable information" when performing preclinical safety testing of therapeutic cancer vaccines and the combination of cancer vaccines with other agents. Considerations for safety studies have been published[73]. The proposal is to focus on using in silico human expression data, and further that it is not feasible to replicate immune responses in animals since immune responses are dependent on human MHC-molecules.

Regarding identification of initial dose, there are likewise no suitable animal models. In the case of peptide vaccination, the dosage should be higher than an unknown threshold level, below which presentation by antigen-presenting cells will not be sufficient. Doses higher than the threshold dose will not increase effectivity, but also do not cause increased toxicity since peptides are rapidly degraded by local peptidases to amino acids. The dosages selected for the current study are based on experience.

In conclusion, studies in animals on safety, characterization of the pharmacokinetics, and determination of maximum tolerated dose (MTD) are not relevant in the case of therapeutic peptide vaccination.

3

Patient recruitment

Patients will be recruited nationally and included at the Department of Oncology at Herlev Hospital. Patients with metastatic melanoma who are candidates to receive Nivolumab monotherapy can be included regardless of what earlier oncological treatment they have received; i.e. surgery, radiation, high-dose IL-2 or experimental treatment.

The majority of patients with metastatic melanoma in Denmark are treated at Herlev, Odense and Aarhus University Hospitals. We expect around 30-40 patients per year to be candidates for monotherapy with Nivolumab. It is therefore estimated that patient inclusion can be completed within 24 months.

Treating doctors at the departments of oncology and plastic surgery can refer patients to the investigator. The investigator reads through the referral material and the patients are then invited to a talk about the protocol at the Department of Oncology at the experimental unit (EFEK).

Information and informed content retrieval

At the first appointment at the experimental unit, patients will be informed orally about the protocol by a doctor with experience in both oncology and immunotherapy under supervision of Professor Inge Marie Svane. The patient is always welcome to bring an assessor (relative, friend etc.) Written patient information in the form of "Participant Information" will be handed out to the patient.
4
The conversation with the participant takes place in an undisturbed room and 60 minutes is allotted.
The information will be presented in an easily comprehensible language without the use of technical or value-laden terms. The consultation and written information will be in Danish, but in cases of foreign languages a certified translator will be provided.

Information given during the consultation will be based on the written participant information and include the following:

- Predictable risks, side effects, possible complications, disadvantages, as well as the possibility for unpredictable risks and complications
- Other treatment options including advantages and disadvantages, as well as the consequences of no treatment and supportive care
- Private and other confidential information contained in the medical record might be reviewed by third party officials during the statutory quality assessment of the trial
- Other conditions which might have influence on the patient's decision on participation
- The possibility for the participant to withdraw their informed consent at any time after inclusion

If the participant wishes to participate, a new appointment will be booked where the consent is signed by the doctor and the participant. The new appointment and time of reflection will be within 4-7 days after the first appointment, but if the patient needs more time of reflection this can also be accommodated. If the patient wishes to sign the content at the first appointment this is also acceptable. If the patient fulfills all in- and exclusion criteria the patient will be included and receive treatment immediately after.

After inclusion, the participant will be informed,

- If additional risks, side effects, complications or other disadvantages are discovered during the trial
- If major changes are made to the protocol which might affect health or safety
- If other relevant health conditions are discovered during the trial so forth the participant has not expressed a wish not to receive such information

- About the results achieved and possible consequences to the patient so forth the participant has not expressed a wish not to receive such information
- If the trial is terminated before schedule, the participant will be informed of the reason

Information related to the participants will be protected under the legislation of processing of personal data and the health legislation, section 3 regarding patient's rights.
Patients will be receiving phone numbers to the "contact center" as well as the doctor on duty at the Department of Oncology. Patients are assigned a "contact doctor" and a "contact nurse" as well as instructions on how to reach these.

Study design

The study is designed as an open phase I/II study. In phase I, 6 patients with metastatic MM will be treated.
5 Around 10-15 % of the patients treated with Nivolumab have grade 3-4 AE.
Before phase II can start, all 6 patients must receive the first 4 treatments without any grade 3-4 adverse events, other than those expected with Nivolumab.
If 3 or more patients experience grade 3-4 AE in phase I associated with vaccination, the trial will be stopped. A minimum of 2 vaccines must be administered before inclusion of another patient for each of the 3 first patients included in the study.

In phase II, additionally 44 patients will be included. All patients will receive the same dose vaccine without dose escalation in combination with Nivolumab. A total of 50 patients will be included.

5.1 Inclusion criteria

1. Age ≥ 18
2. The patient has locally advanced or metastatic melanoma with progressive, persistent or recurrent disease on or following treatment with standard of care agents.
   Patients belonging to one of the following patient groups will be enrolled:
   Cohort A: Anti PD-1/PD-L1 naïve patients (30 patients).
   The patient is a candidate for Nivolumab monotherapy. Prior anti-PD-1/anti-PD-L1 antibody treatment is not allowed. **OR**
   Cohort B: Extension cohort (10 patients). Progressive disease ON anti-PD-1 antibody monotherapy. Subjects should not have experienced serious and/or life-threatening toxicity to antibody therapy. **OR**
   Cohort C: Extension cohort (10 patients). Progressive disease during follow up OFF anti-PD-1 after clinical benefit (SD/PR/CR) on anti-PD-1 antibody therapy. Subjects should not have discontinued antibody therapy due to serious and/or life-threatening toxicity
3. At least one measurable parameter according to RECIST 1.1.
4. The patient has an ECOG performance status of 0 or 1
5. The patient is a female of childbearing potential with negative pregnancy test

6. For women: Agreement to use contraceptive methods with a failure rate of < 1 % per year during the treatment period and for at least 150 days after the treatment. Safe contraceptive methods for women are birth control pills, intrauterine device, contraceptive injection, contraceptive implant, contraceptive patch or contraceptive vaginal ring.
7. For men: Agreement to use contraceptive measures and agreement to refrain from donating sperm
8. The patient has met the following hematological and biochemical criteria:
    a. AST and ALT ≤2,5 X ULN or ≤5 X ULN with liver metastases
    b. Serum total bilirubin ≤1,5 X ULN or direct bilirubin ≤ ULN for patient with total bilirubin level > 1,5 ULN
    c. Serum creatinine ≤1,5 X ULN
    d. ANC (Absolute Neutrophil Count) ≥1,000/mcL
    e. Platelets ≥ 75,000 /mcL
    f. Hemoglobin ≥ 9 g/dL or ≥ 5.6 mmol/L
9. Signed declaration of content after oral and written information about the protocol.

5.2 Exclusion criteria

1. The patient has not recovered to grade 0-1 from adverse events due to prior chemotherapy, radioactive or biological cancer therapy
2. The patient has not recovered from surgery or is less than 4 weeks from major surgery
3. The patient has a history of life-threatening or severe immune related adverse events on treatment with another immunotherapy and is considered to be at risk of not recovering
4. The patient is expected to require any other form of systemic antineoplastic therapy while receiving the treatment
5. The patient has a history of severe clinical autoimmune disease
6. The patient has a history of pneumonitis, organ transplant, human immunodeficiency virus positive, active hepatitis B or hepatitis C
7. The patient requires systemic steroids for management of immune-related adverse events experienced on another immunotherapy
8. The patient has active CNS metastases and/or carcinomatous meningitis. However, patients with subclinical brain metastases < 1 cm can be included (maximum of 4 metastases < 1 cm). (Patients with previously treated brain metastases may participate provided they are clinically stable. Patients with untreated brain metastasis will be excluded)
9. The patient has any condition that will interfere with patient compliance or safety (including but not limited to psychiatric or substance abuse disorders)
10. The patient is pregnant or breastfeeding
11. The patient is unable to voluntarily agree to participate by signed informed consent or assent
12. The patient has an active infection requiring systemic therapy
13. The patient has received a live virus vaccine within 30 days of planned start of therapy
14. Known side effects to Montanide ISA-51

15. Significant medical disorder according to investigator; e.g. severe asthma or chronic obstructive lung disease, dysregulated heart disease or dysregulated diabetes mellitus
16. Concurrent treatment with other experimental drugs
17. Any active autoimmune diseases e.g. autoimmune neutropenia, thrombocytopenia or hemolytic anemia, systemic lupus erythematosus, scleroderma, myasthenia gravis, autoimmune glomerulonephritis, autoimmune adrenal deficiency, autoimmune thyroiditis etc.
18. Severe allergy or anaphylactic reactions earlier in life

5.3 Evaluation before the start of treatment

The following parameters have to be performed within a month of the start of vaccinations (blood tests within a week):

- Medical history and clinical examination
- Performance status according to the ECOG scale
- Toxicity evaluation
- Concurrent medical intake
- Electrocardiogram
- Blood tests:
    - Hemoglobin, leukocyte differentiation count and platelets
    - Potassium, Sodium, Creatinine, albumin, uric acid, lactate dehydrogenase, Alkaline Phosphatase, Alanine transaminase (ALAT), amylase, bilirubin, ionized calcium, C Reactive Protein (CRP)
    - Thyrotropin (TSH), thyroxin (T4), Luteinizing Hormone (LH), Adrenocorticotropic Hormone (ACTH), Cortisol
    - Hepatitis B, hepatitis C (IgG), HIV, HTLV-1(IgG),, Epstein-Barr Virus EBV and Treponema. .
    - Women: Human Chorion Gonadotropin HCG, Follicle stimulating hormone FSH, Estradiol and Prolactin
    - Men: Testosterone
- Pregnancy test: Women of childbearing potential must have a pregnancy test performed at the time of screening. This involves women who are not surgically sterilized, postmenopausal or have used adequate and safe prevention for more than 6 months.
- Diagnostic imaging: PET-CT or CT scan of neck, thorax, abdomen, pelvis and CNS & optionally extremities.

Conduct of the study

7.1 Initial patient enrollment and treatment

A minimum of 2 treatments with the peptide vaccine and Nivolumab has to be administered before inclusion of another patient for each of the first 3 patients included in the study.

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1st patient	1st dose	2nd dose	3rd dose	4th dose	5th dose	6th dose	7th dose	9th dose	10th dose	
Safety evaluation			Go/no go for next patient							
2nd patient				1st dose	2nd dose	3rd dose	4th dose	5th dose	6th dose	
Safety evaluation						Go/no go for next patient				
3rd patient							1st dose	2nd dose	3rd dose	
Safety evaluation before remaining patients' inclusion									Go/no go for remaining patients	
Dosing of remaining patients can start in parallel										X

7.2 Treatment plan and treatment schedule

Patients included in the protocol are treated with Nivolumab according to usual guidelines, implying outpatient IV infusions of 3 mg/kg biweekly until progression. The vaccine is administered on the same day as the administration start of Nivolumab. The vaccination is administered biweekly for a total of 6 times, then every fourth week up to week 47, whereupon no additional vaccinations will be given. In total, 15 vaccines will be administered. A vaccine consists of 100 µg IDO long, 100 µg PD-L1 long1, 25 µl DMSO, 475 µl PBS and 500 µl Montanide. During treatment with Nivolumab a pregnancy test must be done on fertile women every month.

In case of disease progression, patients are referred back to the oncology team, from which they were referred initially. Further treatment after disease progression will not be affected by participation in the study.

	Inclusion																								
Week	**0**	**1**	**3**	**5**	**7**	**9**	**11**	**13**	**15**	**17**	**19**	**21**	**23**	**25**	**27**	**29**	**31**	**33**	**35**	**37**	**39**	**41**	**43**	**45**	**47**
Nivolumab		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Peptide vaccine		V	V	V	V	V	V		V		V		V		V		V		V		V		V		V
Health blood sample	HB	HB	HB	HB	HB	HB	HB		HB		HB		HB		HB		HB		HB		HB		HB		HB
Project blood sample	PB			PB			PB						PB						PB						PB
Toxicity evaluation	TE	TE	TE	TE	TE	TE	TE		TE		TE		TE		TE		TE		TE		TE		TE		TE
Physical examination	PE	PE	PE	PE	PE	PE	PE		PE		PE		PE		PE		PE		PE		PE		PE		PE
PET-CT	PC						PC						PC						PC						PC
Tumor biopsy	TB						TB																		
DTH							X																		

Patients who complete all vaccines will continue Nivolumab treatment after standard guidelines.

Health blood samples:

Nivolumab & Peptide vaccine Females	**Nivolumab & Peptide vaccine Males**
B-Hæmoglobin	B-Hæmoglobin
B-Trombocytter	B-Trombocytter
B-Leukocytter	B-Leukocytter
B-Leukocytfraktioner	B-Leukocytfraktioner
P-Natrium	P-Natrium
P-Kalium	P-Kalium
P-Creatinin	P-Creatinin
P-Carbamid	P-Carbamid
P-Hydrogencarbonat	P-Hydrogencarbonat
P-Albumin	P-Albumin
P-Urat	P-Urat
P-ASAT	P-ASAT
P-ALAT	P-ALAT
P-LDH	P-LDH
P-Basisk Phosphatase	P-Basisk Phosphatase
P-Bilirubin(total)	P-Bilirubin(total)
P-Amalyse	P-Amalyse
P-Glucose	P-Glucose
P-TSH	P-TSH
P-Thyroxin (T4)	P-Thyroxin (T4)
P-Calcium – ion frit	P-Calcium – ion frit
P-Cortisol	P-Cortisol
P-LH	P-testosteron
P-FSH	

P-Estradiol P-Prolactin	

7.4 Follow up

Patients who complete the treatment of vaccines for all 47 weeks will be followed in the specialized unit at EFEK 3 and 6 months after treatment. Patients who have completed the vaccination treatment will continue Nivolumab treatment after usual guidelines in parallel with the follow up.

Month	**3**	**6**
Day:	1	1
Year: 20__		
Toxicity evaluation and performance status	0	0
Clinical examination -	0	0
Health blood samples	0	0
Project blood samples	0	0
PET-CT or CT. scan of neck, thorax, abdomen, pelvis, and possibly CNS and extremities	0	0

Duration of treatment:

Treatment with the PD-L1/IDO peptide vaccine is maximum 47 weeks while treatment with Nivolumab is according to standard treatments, hence as long as there is a clinical effect and the patient tolerates the treatment.

The duration of treatment for Nivolumab on the basis of median progression free survival (mPFS) is 5.5 months corresponding to each patient receiving an average of 11 treatments. The duration will in practice last longer as mPFS may underestimate the proportion of patients with treatment benefit due to early "pseudo" progression prior to response.

Treatment will be stopped if there is a confirmed disease progression according to the immune-related response criteria or by unacceptable toxicity or need for ongoing prednisolone ≥ 10 mg daily.

Concurrent treatment:

Vaccination with live virus vaccines are contraindicated during treatment and initiation treatment must be at least one month after any such vaccinations. Local palliative radiotherapy or surgery of solitary metastases can be performed during treatment.

7.5 Procedures regarding treatment

Patients will be receiving the peptide vaccine & Nivolumab on the same day. Vaccination will be performed first, followed by infusion of Nivolumab.

Peptide vaccine

The vaccine will be administered as a subcutaneous injection of 1 ml on the upper arm altering between the right and left side.

During administration of the first 3 vaccines, the patient is observed for ½ hour with measurements of pulse and blood pressure before and after the vaccine is given. The remainder of observations can be administered with observation of the patient for 15 minutes. Patients are observed for acute toxicities in the form of allergic reactions, including anaphylactic shock. Allergic reactions and/or anaphylactic shock will be treated per hospital guidelines.

The vaccines are ordered after contact to the project nurse at the clinical experimental unit (KFE) and to the manufacturing unit. (SOP: Bestilling og håndtering af vaccine I protokol MM1636, appendix 18)). When the vaccine is received, the "receipt of transport" is signed. (SOP: Transport og udlevering af PD-L1/IDO mix og Montanide, appendix 21).

Nivolumab

Following vaccination and observation, the patient is treated with Nivolumab. Administration of Nivolumab follows the usual guidelines as if the patient was to be treated with Nivolumab alone. This involves IV infusions of a 3 mg/kg dose via a filter dropper over ½ an hour following rinse with isotonic sodium chloride.

Nivolumab is ordered according to usual guidelines with electronic ordering in "Sundhedsplatformen".

If the patient is prevented in attending the planned treatment day, the treatment can be moved to the following Tuesday or Thursday. If the treatment is postponed more than 6 weeks, patients will be excluded.

7.7 Criteria for treatment modifications

Delay of treatment

The treatment will be postponed if the patient experiences non-hematological AE grade ≥ 2, except fatigue. Otherwise the following criteria are applicable:

Blood samples	Criteria
Leukocytes	< 1,5 x 10^9 (grade 3)
Neutrophilic granulocytes	< 1,0 x 10^9 (grade 3)
Thrombocytes	< 50 x 10^9 (grade 3)
Hemoglobin	< 5 mmol/l (grade 3)
Creatinine	>3 x ULM (grade 3)
ASAT	>5 x ULN (grade 3)
ALAT	>5 x ULN (grade 3)
Bilirubin	>3 x ULN (grade 3)
Other AEs	
Skin-related AE	≥ grade 3
All other AE	≥ grade 2

The treatment will be postponed until the severity has been reduced to grade 0 or 1.

Criteria for end of treatment

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1. Unacceptable toxicity
2. Disease progression
3. On patient request
4. Clinical judgement
5. Other treatment
6. Pregnancy
7. Delayed treatment

Unacceptable toxicity:

If treatment related AE grade 3-4 is not reduced appropriately within 12 weeks, the treatment will be stopped and the patient is excluded. Shorter delays can occur multiple times with no consequences for the patient or the trial.
The patient will be excluded subject to the following toxicities:

Blood sample	**Criteria**
Leukocytes	< 1,0 x 10^9 (≥grade 4)
Neutrophilic granulocytes	< 0,5 x 10^9 (≥grade 4)
Thrombocytes	< 25 x 10^9 (≥grade 4)
Hemoglobin	< 4 mmol/l (≥grade 4)
Creatinine	>4,5 x ULM (≥grade 4)
ASAT*	>8 x ULN (≥grade 4)*
ALAT*	>8 x ULN (≥grade 4)*
Bilirubin**	>5 x ULN (≥grade 4)**
Other Adverse Events	
Bronchospasms or hypersensitivity reactions	≥ grad 3
Skin related AE	≥ grad 4
All other AE	≥ grad 3
Any eye pain or reduced visual of grade 2 or above with no restitution within two weeks will lead to exclusion	

*ASAT/ALAT grade 3 with bilirubin > 1,5 x ULN
** Bilirubin > 3 x ULN with ASAT/ALAT > 2,5 x ULN

Disease progression: Treatment is terminated if disease progression occurs at predefined evaluation time points. Pseudo progression where initial tumor growth is seen because of treatment with Nivolumab and actual disease progression is not seen must be considered.

Patient request: The treatment can be stopped at any time if the patient wishes so.

Clinical judgement: The treatment can be stopped because of medical conditions at any time if the investigator finds it in the patients' best interest.

Other treatment: Patients will be excluded at any time a new treatment with an experimental drug or other systemic anticancer treatment is initiated after inclusion in this protocol. The patient will be excluded if systemic treatment with corticosteroids is initiated unless it is on vital indication and in agreement with the physician responsible for the protocol. Supportive treatment is given on usual medical indication and is to be specified in the patient chart and flow sheets.

Pregnancy: A pregnancy test is to be performed before women of childbearing potential can be included in the study. Women of childbearing potential are to contact investigator immediately if pregnancy is suspected (absent or delayed menstruation etc.) at any point during the course of treatment and for 3 months after the end of treatment. A pregnancy test is performed if pregnancy is suspected and the participants can receive no more vaccination treatments and is terminated from the study if it turns out positive. A pregnant participant and the course of the pregnancy will be followed closely in the outpatient clinic.

Delayed treatment: The patient is terminated from the study if the treatment is delayed more than 6 weeks. It is the responsibility of the principle investigator to assess the cause of delay and define the time point for the start of the delay. A patient can be delayed several times during the cause of treatment without being excluded.

8.1 Early termination from the study

Participants, which are excluded from the study, will be referred back to the oncology team from which they came, for standard treatment. Patients that are excluded before the 3rd vaccine will be replaced with new participants.

8.2 Concurrent treatment

- Supportive treatment is given based on clinical judgement and should be noted in the patient charge in accordance with existing practice
- Local palliative radiotherapy is allowed

Manufacture of the peptide vaccine

10.1 Peptides

For detailed description of peptide mixture production see SOP: "Fremstilling af PD-L1/IDO peptid-mix til MM1636" (appendix 16)

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PD-L1 long1

The peptide sequence:
PD-L1 (9-27) F-M-T-Y-W-H-L-L-N-A-F-T-V-T-V-P-K-D-L

IDO long

The peptide sequence:
IDO (194-214) D-T-L-L-K-A-L-L-E-I-A-S-C-L-E-K-A-L-Q-V-F

All amino acid residues are in L-configuration. No disulphide bond is present. The counter ion, hydrochloride is bound in ionic form.
The peptides are manufactured by solid phase synthesis using the Fmoc (9-fluorenylmethyloxycarbonyl) approach. The limit on peptide purity is set to no less than 95 %. The peptides are dissolved in dimethyl sulfoxide (DMSO) and phosphate-buffered saline (PBS). The solution is mixed and filtered through a sterile 0.20 µm filter.

10.2 Producing the peptide vaccine

The final production of the peptide vaccine will take place under GMP approved laboratory conditions (approved by the Danish Medicines Agency) which quality assures a sterile therapeutic product. It will take place in JM702, Herlev and Gentofte Hospital, authority number: 24223.

10.3 Montanide ISA 51

Montanide ISA 51 is produced and sold by Seppic INC: www.seppic.com. France. Montanide is delivered sterile and ready for use in ampules of 3 ml. Montanide ISA 51/Vaccine adjuvant is based on vegetable oil. See product resume or link: http://www.seppic.com/human-health/vaccine-adjuvant/montanide-isa-51-@/1018/view-1042-seproduit.html

10.4 Preparing the vaccine

The peptide mixture and Montanide are delivered to the Department of Oncology shortly before use by a project nurse or laboratory staff from J7. The peptide mixture constitutes 500 µl consisting of 100 µg PD-

L1 long 1, 100 µg IDO long dissolved in DMSO and PBS in a watery solution. Montanide ISA-51 constitutes 500 µl and right before injection the two solutions are mixed through an I-connector luer lock into a 1 ml syringe. The syringe is marked with patient name, personal security number, date and time of preparation. For detailed description of the peptide preparation see SOP "Klargøring ad PD-L1/IDO peptid vaccine til protokol MM1636", appendix 17. See also example of labels for the vaccine in appendix 6.

10.5 Microbiological control

Endotoxin and cultivation tests of the peptide preparations are performed at the Department of Clinical Microbiology, Herlev and Gentofte Hospital before use of the vaccines.

Ordination of Nivolumab

ATC-kode: L01XC17

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Nivolumab is ordered electronically via "Sundhedsplatformen" from the cytostatic unit at Herlev & Gentofte Hospital.
See product resume and link: http://ec.europa.eu/health/documents/community-register/2015/20150717132284/anx_132284_da.pdf.

Nivolumab is labeled in accordance with Annex 13 GMP rules, and a Danish instruction for the staff is established.

Holder of the Marketing Authorization:
Bristol-Myers Squibb Pharma EEIG
Uxbridge Business Park
Sanderson Road
Uxbridge UB8 1DH
Great Britain

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Evaluation

12.1 Data registration

The patients are given a patient number at inclusion in the study to secure patient anonymity. Clinical personal and selected persons in the laboratory will have access to patient information, but only in form of personal ID and name, to secure proper treatment.
The principal investigator has access to patient charts to obtain information regarding the cancer disease to be able to compare this information with the project specific analysis performed on cancer tissue and blood tests.

All relevant data is registered in case rapport form in collaboration with the clinical research unit (KFE). The principal investigator is responsible for manufacturing of the case report form (CRF) and data registration in the CRF after the course of treatment. CRFs will be reported to sponsor. Sponsor and principal investigator are responsible for data analysis on all included participants. Participant data and CRF will be kept for 5 years in accordance with current guidelines for storage of sensitive data. Completion of a final report will be done in collaboration between the members of the project group.

The analysis will include:

1. Adverse events registration
2. Immunological response
3. Clinical endpoints

Sensitive data, and any other remaining samples, will be anonymized at the end of the trial.

12.2 Monitoring

Monitoring of the trial will be done by the GCP unit at the University of Copenhagen. It is expected that the responsible GCP unit will contact and visit the principal investigator on a regular basis and request for access to monitoring of the different source documents in relation to the trial (Case Report Forms and other relevant documents).
Audit from the Danish Medicines Agency and other health authorities are likewise allowed on request. Monitor has access to laboratory test results and other participant information needed to verify the entries made in the Case Report Forms. The principal investigator (or his/her substitute) agrees to cooperate with the monitor so that potential problems discovered during the monitoring process will be solved.

12.3 Statistical considerations

The study is an open phase I/II trial with the primary objective to evaluate safety of the combination therapy. 50 participants are deemed sufficient to evaluate safety and considered safe if less than 20 % experience grade 3-4 AE.

All participants that are included and treated according to the protocol will be included in the statistical analyses. Participants who are excluded before receiving treatment will not be included in the statistical analyses:

- Where the inclusion criteria at baseline are not fulfilled
- Patients withdraw their consent
- Have started other treatment

For immunological responses, the 95 % confidential interval will be calculated for patient data and for response rates descriptive statistics will be used.

12.4 Effect parameters

12.4.1 Primary effect parameter

Primarily, safety and toxicity of the combination therapy is assessed. This is done by registration of all adverse events and possible unwanted events that occur in relation to the treatment and in accordance with the CTCAE criteria. Data collection will take place in accordance with the data source list.

12.4.2 Secondary effect parameter

For the assessment of the immunological response, PD-L1 and IDO specific T cells responses will be identified by the use of ELISPOT technology and antigen specific populations will be characterized for their cytokine secretion pattern using IFN-γ, IL-2 and TNF-α.
We will further examine the effect of the vaccine in different subtypes of immune cells during therapy. Flow cytometry analysis will be performed prior to, during and after treatment. The numbers of different subtypes of immune cells will be registered and compared e.g. memory T cells, Treg, Natural Killer (NK) and MDSC.

12.4.3 Tertiary effect parameter

The clinical effect of the treatment will be assessed by measure of tumor responses by standard imaging (PET-CT) according to PERCIST and RECIST 1.1 as well as irRC, with the endpoints being objective response (OR), progression free survival (PFS) and overall survival (OS).[17]

RECIST
Clinical evaluation will be done in accordance with RECIST 1.1 Guidelines[74].
Complete response (CR): All lesions disappear.
Partial response (PR): Defined as ≥ 30% reduction in the sum of all measurable parameters' longest diameter.
Stabile disease: defined as < 30% reduction in the sum of all measurable parameters' longest diameter or a < 20% increase in the sum of all measurable parameters' longest diameter.
Progression: Defined as a > 20% increase in the sum of all measurable parameters' longest diameter or the appearance of new lesions.
Complete and partial response is to be verified by examination at a minimum of 4 weeks after documentation of the response at the earliest.

Matched controls will be identified using the DMG Oncology MM database to compare PFS and OS. Since September 9th 2015 Pembrolizumab has been 1st line treatment in the majority of patients with MM in Denmark. First, patients treated with Pembrolizumab in the period of 2017-2020 will be identified to collect patients from the same time period as the patients in the protocol treated with Nivolumab and the PD-

L1/IDO peptide vaccine. In 2017 and 2018 a total of 319 patients were treated with Pembrolizumab according to DMG Oncology database.
Controls will be matched according to the following parameters: Age, sex, Performance status (PS), previous treatment, M-stage, PD-L1 status, BRAF status, LDH level, CRP level.
Propensity scores will be conducted before matching the two groups (control vs treatment). A propensity score is the probability that a unit with certain characteristics will be assigned to the treatment group (as opposed to the control group). The scores can be used to reduce or eliminate selection bias by balancing the characteristics of participants between treated and control groups. When the covariates are balanced, it becomes much easier to match participants with multiple characteristics (parameters).
We will collect the necessary data approvals in order to extract the register-based control group.

12.5 Immunologic monitoring

12.5.1 Blood samples for immunological monitoring

100 ml blood will be sampled in Sodium-heparin prepared vials where mononuclear cells are isolated. 8 ml blood will be sampled in a dry medium for serum to be frozen. Project blood samples will be taken 6 times the first year and thereafter every third month up to 5 years.
Immune cells are isolated from the collected blood samples by use of leucosep/lymphopreb-technique and are frozen in a biobank for later analyses; immune assays on viable immune cells. (SOP's: "Isolering af MNC" and "Nedfrysning af MNC" appendix 19 & 20). Antigen specific immune reactivity will be tested by use of a panel of relevant immunological assays including ELISPOT, ELISA, proliferation assays, cytotoxic assays, intracellular staining (ICS), and multimeric staining of PD-L1 and IDO specific CD8 T cells. Blood and serum samples are processed and stored at Center for Cancer Immune Therapy (CCIT).

12.5.2 Tumor biopsy for tumor immune microenvironment evaluation

Efforts shall be made to take biopsies from the available tumor lesions or involved lymph nodes before the 1st vaccine and after the 6th vaccine. Depending on the localization and accessibility, different types of biopsies will be performed. If the involved areas are not directly accessible, the biopsy will be conducted under sterile conditions via ultrasound.
Tumor biopsies will be used for the following assays:

- Immunoscore CR assay will be analyzed performed on the quantification of CD3 and CD8 T cells in the core of the tumor for biopsy samples or in both the margin and the core of the tumor for resected tumors. This is done to asses "Immunoscores" predictive and prognostic value, which in turn will help select the patients who will benefit from the treatment and vice versa in the future.
- Immunoseek CR assay will be analyzed to detect CD8+ lymphocytes and PD-L1+ immune and tumor cells by a multiplexed IHC assay on the same FFPE slid if possible. Standardized Image analysis will allow quantifying cells by digital pathology with the support of a pathologist.
- IHC staining PD-1, will consist in positive PD-1 T cells quantification by digital pathology of a single

FFPE biopsy slide.

- IHC staining IDO will consist in IDO protein quantification by digital pathology on a single FFPE biopsy slide.

As well as gene-based assays:

- Immunosign CR and Pan Cancer Immune profiling on NanoString platform will consist in testing patient sample biopsies for gene expression quantification of different immune genes.
- A whole exome sequencing performed on tumor samples and corresponding healthy tissues on pre-treated patient samples to assess initial mutational status for each patient. [15,16]

The aim of the planned gene analyses is to learn more about;

1. Differences among patients in expression level of a panel of relevant normal genes in the tumor microenvironment which could influence the chance of benefit from treatment.
2. Expression of tumor/patient specific mutated genes which could influence the chance of benefit from treatment.

Analysis for identifying specific tumor gene expression signatures and mutations in the tumor cells, leading to patient-specific neo-antigens derived from these mutations will be performed. These analyses will contribute to identification of patients that are most likely to respond to treatment.

Methods in gen-based assays

A whole exome sequencing will be performed on tumor samples and corresponding healthy tissues on pre-treated patient samples to assess initial mutational status for each patient.

This testing will be performed on Illumina NGS platform per batch of 14 samples.
Total genomic DNA will be extracted from fresh frozen biopsy or from 1 FFPE biopsy block to prepare 1 FFPE slide biopsies of 20µm. The best would be to have fresh frozen biopsies.
If a biopsy of healthy tissue cannot be provided then genomic DNA will be extracted from 5ml whole blood.

Data regarding potential disease causing genes will be generated as a byproduct of this analysis, but this data will not be used or explored further upon, since only data regarding tumor specific genes will be processed more closely. Therefore, we do not expect to obtain explicit knowledge regarding disease causing genes. Furthermore, 'Targeted sequencing' on a limitied number of defined genes will be performed on the tumor tissue to obtain a 'immune profile' to determine which genes- and consequently which proteins- are expressed in the tumor tissue. Data will be handled according to national laws.

If the patient wishes prior genetic counseling by the chance of discovery of mutations in disease genes and/or the risk of random finds this can be accommodated by referral to the Clinical Genetics Department, Rigshospitalet.

If by chance these analyses will discover known mutations with potential significant impact on patient's health, the case will be discussed with the Clinical Genetics Department, Rigshospitalet, unless the patient has chosen not to be informed as stated in the patient information. If the patient wishes genetic counseling it will be offered by specialized doctors, if mutations with potential significant impact on the patient's health are discovered. The following criteria will determine if further actions are indicated.

- There is a reasonable degree of possibility that a genetic disposition is present,
- There is solid documentation of a link between the genetic disposition and the development of disease,
- The tests used to determine the genetic disposition are well established,
- The disease in question can be prevented or treated, and
- The link between the genetic disposition and the development of disease has considerable importance for the patient.

If indicated, the patient will be contacted and asked for permission to referral to such Department for additional information and testing.

In case that a patient dies/is dead, or do not want information regarding significant health issues, a medical assessment, using the 5 above mentioned criteria, will determine whether relatives to the patient is to be informed, in accordance with Danish law (sundhedslovens § 43, stk. 2, nr.2.).

As described above, we will perform DNA analyzes to clarify the function of the patients' immune system. These analyzes will be done by Adaptive Biotechnologies. Data will be stored pseudonymized and encrypted by Amazon Web Services and Microsoft Azure (both cloud data centers in the United States). Data will not be deleted from the cloud, but pseudonymization and encryption protects the patient's data. Only those responsible for this study have access to these clouds. The Danish data protection agency has approved Adaptive biotechnologies as a partner in the trial.
Patients will be informed either by physical attendance or by telephone. They will have time to think and can always retreat. Patients will sign a new consent and they have the opportunity to ask questions both before signing and afterwards.

12.5.3 Delayed type hypersensitivity (DTH) & biopsy

Delayed type hypersensitivity will be tested after 6 vaccinations. The test consists of up to a total of 3 intradermal injections of either an aqueous solution of PD-L1/IDO or the negative control saline. After 48 hours, the diameter of the reaction is measured, and 6mm punch biopsies are taken from the injection sites. The biopsies are divided in two, half is frozen for later investigation, half is used to grow skin-infiltrating lymphocytes (SKILs). SKILs are tested for specificity to the PD-L1/IDO peptides as a sign of

induction of a functional immune response. The patient can refuse to undergo the DTH-testing without exclusion of the trial.

12.6 Biobank and handling of biologic material

In connection with the current study, blood samples (110 ml/blood sample) and tumor biopsies will be stored in coded form at -150 °C in a research biobank at the CCIT in room PA102 until all analysis concerning the study is performed. Blood samples will be taken up to 5 years after inclusion. The maximum duration of the research biobank will therefore be 7 years (i.e until 2024) (i.e. the last person included is still alive after 5 years). The material is hereafter transferred to a biobank for future biomedical research for up to 15 years if accepted by the Danish Data Protection Agency, after which the remaining material will be destroyed.

Analyses will be primarily performed at CCIT. However, some special analyses on tumor tissue or blood test samples will be performed at a partner institution after establishing a specific written agreement. All patient relevant information will be sent in an anonymous way. In case the patient's cells will be sent to partner institutions located abroad, these will be handled according to national laws and regulations of the specific nation where these have been sent. In such a case, all patient information will be communicated in coded form. A written data processing agreement will be signed between the data controllers and the data processors abroad. If any gene analysis are to be performed abroad, the data processing agreement will include the 5 criteria (See 12.5.2) regarding the discovery of known mutations with potential significant impact on patient's health, as well as the requirement, that the partner abroad reports back to the primary project managers in Denmark so that relevant actions can be taken as described in section 12.5.2. If data processing is to be performed in a third-country, permission will be applied for at the Danish Data Protection Agency, or one of the agencies standard contracts will be used.

Samples that are not used in the study will be transferred to a biobank at CCIT for future biomedical research for up to 15 years and if accepted by the Danish Data Protection Agency. If additional studies in other research areas are to be performed on any samples obtained during the conduct of this study/samples are transferred to a new biobank, a request to do so will be submitted to the Scientific Ethics Committee, Capital Region of Denmark, as per the 'Act of Processing of Personal Data' §§ 5 and § 10, paragraph 2 and 3. After 15 years, any remaining tissue samples will be disposed of according to the local guidelines for destruction of biohazardous waste.
If a patient withdraws his/her informed consent, all biological material is to be destroyed if the patient wishes so.

12.7 Risk evaluation

Evaluation of potential risks associated with the peptide vaccines are based on clinical experiences from other cancer vaccination trials. The risks associated with the immune modulating antibody Nivolumab are well known. Combination therapy with an immune modulating antibody and a peptide vaccine is also based on experience from other combination trials.

Acute reactions

Subcutaneous injections can in theory cause anaphylactic reactions as any other vaccine, but so far it has not occurred for cancer vaccines.

The kinetics of immune induction by cancer vaccines is slow, - that is weeks to months as compared to check point inhibitors.

Autoimmunity

Another theoretical risk is the possibility of inducing autoimmunity when/if the vaccine eliminates the immunosuppressive PD-L1 and IDO positive cells. PD-L1 specific and IDO specific T cells are present in unvaccinated healthy individuals as well as cancer patients. Vaccination with PD-L1 and IDO peptides boosts the activity of these T cells to engage cancer cells and immune cells who express PD-L1 or IDO as part of the local immunosuppressive microenvironment in cancerous tissues. Normal non-cancerous tissues express PD-L1 to some degree, and this expression is increased in the case of inflammation. This is contrasted with cancerous tissues where PD-L1 and IDO is chronically overexpressed. The difference in expression may provide the therapeutic window allowing PD-L1 specific T cells to discriminate cancer cells and their tumor-associated immunosuppressive cells from normal cells. When activated, PD-L1 specific T cells will themselves upregulate PD-1. This means that the activation of the immune response against PD-L1 expressing cells will be self-limiting.

However, in patients with autoimmune diseases or chronic inflammation, PD-L1 and IDO might play a dampening role, thus these patients are excluded.

Autoimmune reactions are well-known side effects to PD-L1/PD-1 inhibitors with pneumonitis being the most severe. Due to 3 cases of death related to pneumonitis in the early trials of the PD-1 inhibitors, specific guidelines for early identification and intervention has been developed, which will be followed in this trial. These guidelines have successfully reduced the severity of AEs.[75]

The damage by autoimmune reactions depends on which organ is involved and to which degree. Endocrine disturbances can occur permanently but mostly transiently in the thyroid gland, hypophysis, adrenal gland and rarely the pancreas. For these disturbances substitution therapy is available for the duration of the disturbances.

Grade 1 and 2 autoimmune reactions can in most cases be reversed with anti-histamines or local corticosteroids without discontinuation of the trial drugs, but higher grade events will lead to discontinuation and exclusion.

Tolerance

In contrast to autoimmunity there is also a theoretical risk of tolerance development leading to increased immune suppression and tumor tolerance and thus increased disease aggressiveness. However, this phenomenon is purely theoretical and the eventual risk is unknown.

Procedures and examinations

Patients included in the trial will have additional blood tests performed compared to patients receiving standard treatment. The amount of blood and the specific procedure when taking a blood sample are not associated with any significant risk. There is a minimal risk of bleeding and infection and pain and bruising can occur in the area.

Tumor biopsies will be taken by experienced doctors, and is not associated with any significant risk; again there is a small risk of bleeding and infection and pain and bruising might also accur in the area.

PET-CT scans are not considered a risk based on the low dose radiation. A full body PET-CT with FDG gives a radiation dose of around 12 milliSievert (mSv), which is about 5 times the annual background radiation. The amount of PET-CT scans in this trial is the same as if the patient was to be treated with standard treatment. The contrast used can very rarely cause allergic reactions and brief reduction in renal function, therefore patients are screened by standard procedures prior to the examination.

Combination therapy

Combination therapy is becoming more and more common. As mentioned earlier, combining Nivolumab and Ipilimumab is just approved as first line standard treatment in MM to patients with low expression of PD-L1. In the studies of monotherapy with Nivolumab around 10-15 % patients experienced grade 3-4 t AE. When combining PD-1 and CTLA-4 blocking antibodies, grade 3 or 4 toxicity is seen in around 53 % of patients, thus being quite toxic.

The IDO peptide vaccine in combination with Ipilimumab showed no grade 3-4 AE.[12] PD-L1 is like IDO an immune regulatory molecule with a similar pattern of expression, and combining Nivolumab with the PD-L1/IDO peptide vaccine is believed to induce toxicity at the same level as if the patient was to be treated with Nivolumab alone.

This statement is based on other studies where combination with an immune regulatory antibody (Ipilimumab or Nivolumab) and a peptide-based vaccine did not result in increased toxicity of signifcance compared to monotherapy of either Ipilimumab or Nivolumab.

Following studies supports the statement. See reference:

- **(Gibney et. Al., 2015)** [76]
    - 30 patients with metastatic melanoma were treated every second week with **Nivolumab** and the **multi-peptide vaccine** (gp100, MART-1, NY-ESO-1 with Montanide ISA 51 VG) for 12 doses followed by maintenance Nivolumab every 12 weeks for 8 doses. Nivolumab in combination with a multi-peptide vaccine was well tolerated. The common adverse events

seen in this trial were similar to past studies with Nivolumab alone. The addition of vaccine led to grade 1 local injection site reactions in a majority of patients.

- **(Bjoern et al., 2016)**[12]
  - **IDO long peptide** has been tested in combination with the anti-CTLA4 check-point blocking antibody **Ipilimumab** in 10 patients with MM in a first in man phase I study. [12] There were minimal toxicity and no grade 3-4 toxicities which could be associated with the IDO vaccine. The majority of patients experienced mild to moderate injection site reactions at the vaccine site, including itching, swelling and erythema. In most cases symptoms responded to oral antihistamines and symptoms remitted after completion of vaccine treatment in all patients. As previous reported to DKMA. One patient developed presumed ipilimumab-induced colitis, which initially responded to corticosteroids, but later relapsed while the patient due to unfortunate circumstances was admitted to a local hospital where the patient died after receiving suboptimal therapy. Colitis is a known severe side effect to ipilimumab that requires intensive management.
- **(Hodi et al., 2010)**[25]
  - In this phase 3 study 676 patients were treated with either Ipilimumab alone (137), peptide vaccine consisting of the gp100 peptide alone (136) or **Ipilimumab plus gp100 peptide** (403). The addition of the gp100 peptide vaccine led to grade 1 local injection site, but the common adverse events where similar with the ones seen with Ipilimumab alone.

All in all, combination of a peptide vaccine (both consisting of multi peptides or one peptide) and an immune regulatory antibody (Ipilimumab or Nivolumab) have not been reported to lead to increas severe toxicity as compared to monotherapy with check point inhibitors.

13 Monitoring of toxicity

13.1 Adverse events

Adverse events (AE) are defined as any undesirable experience occurring to a subject during a clinical trial, whether or not considered related to the investigational treatment. All AEs reported spontaneously by the subject or observed by the investigator or his staff will be recorded and described in the patient chart and the electronic Case Report Form (eCRF). The severity and consequences will be recorded for each AE. The severity and relation to the study medication will be assessed in accordance with the guidelines described in the following.
The investigator must attempt to identify all clinical and objective events from patients receiving treatment and determine their relation to the study medication. The investigator determines the relationship between AEs and treatment using the following guidelines:

13.2 Grading of adverse events

The severity of an AE refers to the intensity of the reaction.

Events are graded using CTCAE version 4.0 (Appendix 1). The following scale can be used if this grading is not applicable:

1 = light
2 = moderate
3 = severe
4 = life threatening
5 = lethal

Patients experiencing AEs will be monitored with the relevant clinical evaluations and laboratory investigations assessed by the attending physician. All AEs must be monitored until satisfactory restitution or stabilization. Results of the monitoring must be recorded in the patient chart and eCRF.
Abnormal laboratory tests are not to be recorded in the eCRF unless they have caused a clinical event, resulted in termination of the treatment or otherwise meet the criteria of a serious AE (see the following).

13.3 Serious adverse events

A serious adverse event (SAE) is to be reported to sponsor within 24 hours and is defined as any medical occurrence or effect that occurs at any dose:

- results in death;
- is life threatening (at the time of the event);
- requires hospitalization or prolongation of existing patients' hospitalization;

- results in persistent or significant disability or incapacity;
- leads to a congenital anomaly or birth defect;
- is a significant medical event

13.4 Guidelines for adverse events' possible relation to the treatment

0 No relation– no temporal relation, other etiologies very likely the cause
1 Possible relation – less clear temporal relation, other etiologies likely the cause
2 Probably related – clear temporal relation with recovery at termination of treatment, and not reasonably explained by the patient's known clinical condition
3 Related – clear temporal relation with laboratory confirmation or a positive retreatment test

13.5 Adverse reactions

An adverse reaction (AR) can be expected if described in the IMPD or the relevant product summary, or unexpected if the grade or severity does not correlate with the product information in the before mentioned documents.
If the AR is unexpected, meets the criteria of a serious adverse reaction (SAR) and is found related to the investigational treatment, it is classified as a suspected unexpected serious adverse reaction (SUSAR)

13.6 Reporting of adverse events and adverse reactions

Investigator reports SAEs, SARs and SUSARs to sponsor within 24 hours. Sponsor reports SUSARs to the Danish Medicine Agencywithin 7 days if considered life threatening or fatal, and otherwise within 15 days. Consequences for the study must be reported. Sponsor submits a list annually that summarizes any SAEs and SUSARs as well as a report regarding the study patients' safety to the Danish Medicine Agency and the Research Ethics Committee (investigator can report to the Research Ethics Committee as well).
Sponsor submits a final report to the Danish Medicine Agency at the end of the study, with a description of all SAEs, SARs and SUSARs.

The following is not to be reported:

- deaths caused by the malignant disease or progression
- hospitalizations or prolongation of current hospitalization caused by the malignant disease:
    - weight loss
    - fatigue
    - electrolyte derangement
    - pain management
    - anxiety

  - palliative hospitalization
  - stay at hospice or terminal care
  - progression of the underlying disease
- hospitalizations or prolongation of current hospitalization if the sole reason for hospitalization or prolongation is:
  - fluid treatment or treatment of nausea
  - blood transfusion
  - platelet transfusion
  - febrile leucopenia/neutropenia
  - administration of investigational procedures
  - placement of a permanent intravenous catheter

These events are to be registered in the eCRF.

13.7 Known adverse reactions

13.7.1 Montanide ISA-51

Montanide ISA-51 has been used in more than 150 clinical trials. Montanide has a well-documented safety-profile. Frequently reported side effects after injection are flu-like symptoms (headache, chills, fever and nausea) while most common local reactions are granuloma, local pain, inflammation or erythema. These reactions are generally classified as mild to moderate and they are typically transient in nature.

Reference is made to attached drug information leaflet. (Appendix 12)

13.7.2 IDO long peptide

The IDO long peptide has already been tested in patients with MM in combination with the immune modulating antibody Ipilimumab. The vaccine was well tolerated with no grade 3-4 toxicity. Most patients experienced mild to moderate reactions at the site of injection, i.e. erythema, inflammation and itching. In most of the cases, symptoms could be treated with oral antihistamine and were typically transient in nature. [12] Reference is made to attached IMPD. (Appendix 5)

13.7.3 PD-L1 long1 peptide

PD-L1 long1 peptide is currently being tested in a phase I first-in-man trial to patients with multiple myeloma. (EudraCT number 2016-000990-19) Data regarding toxicity does not exist yet.
Firstly, all previous trials with peptide vaccinations have very mild side effects, no matter which proteins have been targeted, as reviewed by Rahma and colleagues.[77] Secondly, vaccination against PD-L1 is in many ways similar to vaccination against IDO, and there are no apparent reasons why the toxicity should be higher when vaccinating against PD-L1. Both PD-L1 and IDO are self-proteins with broad expression in

inflamed or cancerous tissues but low expression in healthy tissues. Both are lymphocyte-suppressive molecules and immunosuppressive cancer-associated immune cells. The adverse events in our trials with IDO vaccination have been at the same low levels as that of other peptide vaccines.
In exploratory MOA murine studies, the human sequence as expected does not induce activation of PD-L1 specific T cells in mice, whereas, vaccination with the murine sequence induces strong responses. No toxicity was found in mice vaccinated with either human PD-L1 long1 & murine PD-L1 long1.
Studies on animals on safety, characterization of the pharmacokinetics, and determination of maximum tolerated dose (MTD) are not relevant in the case of therapeutic peptide vaccination. (See section 2.12)
Reference is made to attached IMPD. (Appendix 5)

13.7.4 Nivolumab

As described earlier, Nivolumab exerts its effect on T cells by blocking PD-1 whereby the T cells become more activated and able to kill the tumor cells.
Due to these mechanisms, immune-related AE (irAE) can arise, which in most cases are mild, but can evolve to be serious and require treatment. Grade 3-4 adverse events are reported in approximately 10-15 %. Half of the side effects involve the gastrointestinal tract, the endocrine glands, the liver, lungs and the skin. The AE rarely arise acute, but can be serious/life-threatening if not treated in time. The side effects are generally manageable and the patients are observed closely by an experienced staff at the Department of Oncology.
Reference is made to attached drug information leaflet for Nivolumab. (Appendix 13)

14 Handling adverse events

Patients will be admitted at the Department of Oncology, Herlev Hospital, in case of occurrence of adverse events related to the investigational treatment that requires hospitalization.
The intensive care unit at Herlev Hospital will be informed of the study, since intensive care support could potentially be required if a treated patient develops anaphylactic shock. The information will be given prior to the start of patient inclusion in the protocol and treatment will not begin before the department management at the intensive care unit has confirmed that the information is received.

Insurance

Patients' participation in the study will be covered by the hospital's liability insurance.

Ethical aspects

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This project will potentially add to existing knowledge in the field of immune therapy and help improve treatments and thus prognosis of MM. First line standard treatment for patients with malignant melanoma is both Ipilimumab and Nivolumab in combination or Pembrolizumab/Nivolumab alone depending on PD-L1

16 expression. Patients treated in this trial are PD-L1 positive patients. Addition of a peptide vaccine to the existing standard treatment is believed to add no further toxicity. An earlier trial combining Ipilimumab with an IDO peptide vaccine showed no grade 3-4 AE.

There does not seem to be unacceptable risks or disadvantages regarding the planned treatment based on the current knowledge on peptide vaccines and combination with immune modulating antibodies.

Participation is voluntary and is preceded by oral and written information and the treatment will be stopped in case of unacceptable adverse reactions or if the patient wishes so at any time. The patient will receive treatment after the current guidelines at the department or referring department if he/she does not wish treatment according to the protocol. The study is therefore assessed as ethically proper.

The study follows the Helsinki agreement and the principal investigator is to obtain permission from the Danish Medicine Agencyand the Research Ethics Committee.

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Reporting to the Danish Data Protection Agency

The study is reported to the Danish Data Protection Agency when approvals from the Danish Medicine Agency and the Research Ethics Committee are obtained. The law dealing with personal data will be respected. Information related to study patients is protected according to the law on personal data and the Act on Research Ethics Review of Health Research Projects.

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End of study report

Sponsor will inform the Danish Medicine Agency and Research Ethics Committee within 90 days of study completion. The definition of study completion is 6 months after the last patients' treatment or after exclusion due to progression. In addition, patients will be followed for PFS and OS for up to 5 years. If the study is prematurely terminated, the Danish Medicine Agency will be informed of the reason(s) for the termination. Sponsor will submit a final study report to the Danish Medicine Agency and the Research Ethics Committee with the study results including publications based on the study within a year of study completion.

Publications

Cathrine Lund Lorentzen, Julie Westerlin Kjeldsen, Mads Hald Andersen, and Inge Marie Svane constitute the group responsible for the project.

Under the circumstance that the Vancouver rules are met, the group responsible for the project holds equal rights to the achieved results. The use and presentation of the data in any form, orally or written, can only take place with accept from everybody in the group. Positive, negative and inconclusive results will be reported in scientific journals. The order of authorship is determined by effort and the investigators will be co-authors on further publications derived from this study. The main analysis will be done when all patients have been treated which is expected to be during the year 2018/19.

Economy

The trial is fully financed. Funding has been acquired partly through the Department of Oncology and through IO Biotech ApS. IO Biotech is a biotech firm established in the light of peptide vaccine patents from CCIT and "Region Hovedstaden". Lundbeck, Novo and Sunstone have invested in IO Biotech. The project is co-funded through a research agreement between the capital region and IO Biotech with 2.500.000 Danish crowns over 3 years. The money will be transferred to a research account with a doctor salary of 3 years and 700.000 Danish crowns for immune analyses for the vaccinated patients. IO Biotech also finances the vaccine products, while the remaining expenses are covered by CCIT and the department of oncology.

The project has been initiated by Mads Hald Andersen, Inge Marie Svane, Julie Westerlin Kjeldsen, and Cathrine Lund Lorentzen.

The PD-L1 and IDO vaccine is developed by Mads Hald Andersen who is a part of the management at the CCIT. By Danish law on public inventions at public institutions, the capital region holds the patent, which is licensed for commercialization through the industrial partner IO Biotech. In the case of a sale of the patent, by law the income will be distributed with 1/3 for the inventor, 1/3 for CCIT, 1/6th to Herlev and Gentofte Hospital and 1/6th to Tectra, the technology transfer unit of the capital region.

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