

**PD-L1 PET imaging in patients with melanoma or NSCLC with brain metastasis and eligible for treatment with nivolumab.**

**PROTOCOL TITLE: PD-L1 PET imaging in patients with melanoma or NSCLC with brain metastasis and eligible for treatment with nivolumab.**

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**TABLE OF CONTENTS**

1. SUMMARY .....	9
2. INTRODUCTION AND RATIONALE.....	11
2.1 Immune checkpoint inhibition.....	11
2.2 PD1/PD-L1 blockade in melanoma and non-small cell lung cancer .....	11
2.3 Molecular imaging with [18F]PD-L1 .....	12
2.4 Rationale .....	13
3. OBJECTIVES.....	13
3.1 Phase one .....	13
3.2 Phase two.....	13
4. STUDY DESIGN.....	14
5. STUDY POPULATION.....	14
5.1 Study population .....	14
5.2 Inclusion criteria .....	15
5.3 Exclusion criteria .....	16
5.4 Sample size calculation.....	17
6. TREATMENT OF SUBJECTS.....	17
6.1 Investigational product/treatment.....	17
6.2 Use of co-intervention.....	17
7. INVESTIGATIONAL PRODUCT .....	18
8. NON-INVESTIGATIONAL PRODUCT .....	19
8.1 Name and description of non-investigational product(s) .....	19
8.2 Summary of findings from non-clinical studies .....	19
8.3 Summary of findings from clinical studies .....	19
8.4 Summary of known and potential risks and benefits .....	19
8.5 Description and justification of route of administration and dosage .....	19
8.6 Dosages, dosage modifications and method of administration .....	19
8.7 Preparation and labelling of Non Investigational Medicinal Product .....	19
8.8 Drug accountability .....	19
9. METHODS .....	20
9.1 Primary study parameter/endpoint .....	20
9.2 Secondary study parameters/endpoints .....	20
9.3 Randomisation, blinding and treatment allocation.....	20
9.4 Study procedures.....	20
9.4.1 Patient evaluation .....	20
9.4.2 PET procedure and quantification of tracer uptake .....	21
9.4.3 CT scan.....	23
9.4.4 MRI scan.....	23
9.4.5 Biopsy .....	23
9.4.6 Immunohistochemistry .....	23
9.5 Withdrawal of individual subjects .....	24
9.6 Replacement of individual subjects after withdrawal .....	24
9.7 Premature termination of the study.....	24

10.	SAFETY REPORTING .....	25
10.1	Section 10 WMO event.....	25
10.2	AEs, SAEs and SUSARs .....	25
10.2.1	Adverse events (AEs).....	25
10.2.2	Nonserious adverse events .....	25
10.2.3	Serious adverse events (SAEs) .....	25
10.2.4	Suspected unexpected serious adverse reactions (SUSARs) .....	27
10.3	Annual safety report .....	29
10.4	Follow-up of adverse events .....	29
11.	STATISTICAL ANALYSIS .....	30
11.1	Primary study parameter(s) .....	30
11.2	Secondary study parameter(s).....	30
12.	ETHICAL CONSIDERATIONS.....	32
12.1	Regulation statement.....	32
12.2	Recruitment and consent.....	32
12.3	Objection by minors or incapacitated subjects (if applicable) .....	32
12.4	Benefits and risks assessment, group relatedness.....	32
12.5	Compensation for injury .....	33
12.6	Incentives (if applicable) .....	33
13.	ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION .....	34
13.1	Handling and storage of data and documents .....	34
13.2	Monitoring and Quality Assurance .....	34
13.3	Amendments .....	35
13.4	Annual progress report.....	35
13.5	End of study report .....	35
13.6	Public disclosure and publication policy.....	35
14.	STRUCTURED RISK ANALYSIS.....	36
14.1	Potential issues of concern .....	36
14.1.1	Level of knowledge about mechanism of action .....	36
14.1.2	Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism.....	36
14.1.3	Can the primary or secondary mechanism be induced in animals and/or in <i>ex-vivo</i> human cell material?.....	36
14.1.4	Selectivity of the mechanism to target tissue in animals and/or human beings .....	36
14.1.5	Analysis of potential effect .....	36
14.1.6	Pharmacokinetic considerations .....	37
14.1.7	Study population .....	37
14.1.8	Interaction with other products.....	37
14.1.9	Predictability of effect .....	37
14.2	Synthesis .....	37
15.	REFERENCES .....	38

**LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS**

<b>ABR</b>	<b>ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)</b>
<b>AE</b>	<b>Adverse Event</b>
<b>AR</b>	<b>Adverse Reaction</b>
<b>CA</b>	<b>Competent Authority</b>
<b>CCMO</b>	<b>Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek</b>
<b>CTLA-4</b>	<b>Cytotoxic T-Lymphocyte-associated protein-4</b>
<b>CV</b>	<b>Curriculum Vitae</b>
<b>DSMB</b>	<b>Data Safety Monitoring Board</b>
<b>EU</b>	<b>European Union</b>
<b>EudraCT</b>	<b>European drug regulatory affairs Clinical Trials</b>
<b>FFPE</b>	<b>Formalin fixed-paraffin embedded</b>
<b>GCP</b>	<b>Good Clinical Practice</b>
<b>IB</b>	<b>Investigator's Brochure</b>
<b>IC</b>	<b>Informed Consent</b>
<b>IHC</b>	<b>Immunohistochemistry</b>
<b>IMP</b>	<b>Investigational Medicinal Product</b>
<b>IMPD</b>	<b>Investigational Medicinal Product Dossier</b>
<b>METC</b>	<b>Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)</b>
<b>PD-1</b>	<b>Programmed Death Receptor-1</b>
<b>PD-L1</b>	<b>Programmed Death Ligand 1</b>
<b>PK</b>	<b>Pharmacokinetic</b>
<b>ROI</b>	<b>Region-of-interest</b>
<b>(S)AE</b>	<b>(Serious) Adverse Event</b>
<b>SPC</b>	<b>Summary of Product Characteristics (in Dutch: officiële productinformatie IB1-tekst)</b>
<b>Sponsor</b>	<b>The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.</b>

<b>SUSAR</b>	<b>Suspected Unexpected Serious Adverse Reaction</b>
<b>Wbp</b>	<b>Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgegevens)</b>
<b>WMO</b>	<b>Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen)</b>
<b>WOCBP</b>	<b>Women of child bearing potential</b>



## 1. SUMMARY

**Rationale:** Clinical trials have shown efficacy of PD1/PD-L1 checkpoint inhibitors in multiple solid tumors, including melanoma. Whole body information with regard to target presence, drug kinetics and dynamics, as well as binding of PD-L1 targeting agents to the immune system cells is lacking. Molecular imaging of PD-L1 could lead to new insights on heterogeneity of PD-L1 expression in metastatic lesions and be of help in the prediction of response to PD1/PD-L1 inhibitors in a non-invasive manner.

**Primary objectives:**

Phase one: To assess the dynamics and kinetics of [18F]PD-L1 in human subjects to establish optimal scan schedule.

Phase two: To validate the [18F]PD-L1 PET tracer by association of PD-L1 tumor expression as determined on PET and PD-L1 expression as determined by immunohistochemistry and to assess the association between the radiological response to anti-PD1 treatment and PD-L1 expression as measured by PET scanning and by immunohistochemistry

**Secondary objectives:** To determine between-subject and within-subject variability in [18F]PD-L1 uptake in melanoma and non-small cell lung cancer metastases

**Study design:** This is a feasibility study for the use of [18F]PD-L1 as a PET tracer that will be conducted in a single center. The study consists of two phases. The aim of phase one is to provide pharmacokinetic information on the tracer and to determine the optimal time point for imaging. In the second phase the main study objective will be assessed.

**Study population:** The study population consists of patients diagnosed with metastatic melanoma or non-small cell lung cancer that are eligible for treatment with nivolumab, a PD-1 blocking monoclonal antibody. Patients must be at least 18 years of age. In total, a maximum of 15 patients will be included.

**Intervention (if applicable):** The main intervention of this study is a [18F]PD-L1 PET scan. In both phase one and phase two a scan sequence will be performed both at baseline and 6 weeks after initiation of nivolumab treatment. The PET scans will be combined with either a low dose or diagnostic CT scan of chest, abdomen and pelvis and a MRI of the brain. In phase two, a biopsy of at least one accessible lesion will be performed to analyze PD-L1 expression using immunohistochemical staining after each PET scan.

**Main study parameters/endpoints:**

- Phase one: To determine the optimal schedule for performing [18F]PD-L1 PET scans.

- Phase two: To assess the association of PD-L1 expression as measured by PD-L1 tracer uptake on PET with PD-L1 expression as measured by immunohistochemical (IHC) staining for PD-L1 of corresponding tumor lesions.

**Nature and extent of the burden and risks associated with participation, benefit and group**

**relatedness:** The expected applied injected activity of [ $^{18}\text{F}$ ]PD-L1 is 200 MBq, as is the case for most other  $^{18}\text{F}$ -labeled tracers. The radiation burden for a dose of 200 MBq is approximately 4.4 mSv. Patients will receive 2 serial PET scans each accompanied by a low dose CT scan which adds up to a total dose of 11.8 mSv. The radiation burden of these patients complies with category III (moderate risk) according to the International Commission on Radiological Protection (ICRP) guidelines (ICRP publication 62).

The biopsies needed in phase two of this study are obtained using an invasive procedure. Although biopsies are generally considered to be safe, they can be painful and carry certain risks, such as bleeding at the puncture site. Biopsies will preferably be taken from easily accessible lesions to minimize the burden and risk for the patient.

Whenever possible, all procedures that are part of the study protocol will be planned during regular visits to the hospital as part of care as usual.

## 2. INTRODUCTION AND RATIONALE

Melanoma is the most aggressive form of skin cancer. Its incidence has grown fast over the past few decades, with a current worldwide incidence rate of 121.000 and a mortality rate of 31.000 per year (1). In recent years important advancements have been made in the treatment of metastatic melanoma. One of these was the discovery that cancer could escape immune surveillance by expression of inhibitory immune checkpoint molecules, such as CTLA-4 and PD-1/PD-L1.

Lung cancer is the leading cause of cancer mortality worldwide for both men and women. The main risk factor for the development of NSCLC is smoking. In 2012 about 800 patients died of melanoma and over 10,000 people died of lung cancer in the Netherlands (2). During the last few years, important advancements have been made in the treatment of locally advanced and metastatic melanoma and NSCLC. One of them is the discovery and use of immune checkpoint inhibitors as successful therapy in these patients.

### 2.1 Immune checkpoint inhibition

For T cell activation two signals are necessary: binding of the antigen (AG) presented by the major histocompatibility complex to the T-cell receptor (TCR), and costimulatory signals from antigen-presenting cells (APCs) (3). Whereas co-stimulatory pathways will enhance T cell activation, proliferation and differentiation, so called co-inhibitory signaling pathways, such as the PD-1/PD-L1 axis, will counteract these effects (3,4). Upregulation of these so called checkpoint molecules promote attenuation of T cell activation (3). Under normal physiological circumstances, these mechanisms regulate tolerance by suppressing the immune response and thus protecting the body against self-reactive T cells and controlling inflammation within target organs. Tumors take advantage of these and other mechanisms to evade host immune responses and silence the immune system.(3,4-8) To overcome the functional unresponsiveness of the immune system and subsequently induce tumor regression, checkpoint inhibitors targeting CTLA-4, and more recently inhibitors targeting PD1/PD-L1, have been developed and successfully been tested in several solid tumor types with durable responses in a subset of patients (3).

### 2.2 PD1/PD-L1 blockade in melanoma and non-small cell lung cancer

Current first line therapy for patients with unresectable melanoma consists of treatment with anti-PD-1 immunoglobulines such as nivolumab (BMS-936558) or pembrolizumab (MK-3475). Both agents have shown an increase in overall survival in phase III studies, with preferable toxicity profiles as compared to other agents, such as the CTLA-4 inhibitor ipilimumab.

Data from the phase 1 trial for pembrolizumab showed a confirmed response rate in 38% of the patients (9). Objective response rate in a phase 2 trial of nivolumab was 32% (10). Nivolumab led to an even more durable response in patients with stage IV melanoma than ipilimumab, with a median overall survival of almost 17 months compared to 10 months with ipilimumab. In addition, the safety profile of nivolumab is better than that of ipilimumab, with fewer grade 3 or 4 adverse events.

Because of their different mode of action and different phase of T cell activation in which they play a role, combining nivolumab and ipilimumab has been explored in a clinical trial. This combination of CTLA-4 and PD1 blockade led to even higher objective response rates of up to 53%. Recently, this combination therapy has been approved in the Netherlands for the treatment in unresectable melanoma patients. However, the increase in response rates comes at the cost of more toxicity than either single agent (11).

Although efficacy data on immune checkpoint inhibitors are very promising, not all patients will benefit from these agents. Also, immune-related adverse events (irAEs), especially in combination therapy, can limit the use of immune modulating drugs. Key issues in the future of immunotherapy are the understanding of drug kinetics and dynamics in vivo, as well as the effects of these agents on the immune system, since these may be of help in the selection of patients that will benefit from checkpoint inhibiting therapy.

The objective response (ORR) rate of anti-PD-1 therapy in NSCLC varies based on histological subtype and PD-L1 expression of the tumor. For squamous cell and planocellular carcinoma with low PD-L1 expression ORR are 17% and 9% respectively (12,13). For low-PD-L1 adenocarcinoma, ORR is 8%. High PD-L1 expressing tumours show ORR rates of 30 to 45% (14, 15, 16).

between 19.4 – 42.5%, depending on PD-L1 expression (17). Compared to docetaxel, anti-PD-1 therapy showed prolonged survival (10.4 to 12.7 months vs. 8.5 months) (18). Overall, the treatment is well tolerated, with 9,5% adverse events of grade 3 or higher (17). Anti-PD-1 therapy was approved by the FDA for second line treatment of patients with high PD-L1 expressing NSCLC (>50 % based on IHC assay) in 2015 (16). Recently the indication was expanded to patients with tumors that express  $\geq 1\%$  PD-L1 (18).

### **2.3 Molecular imaging with [18F]PD-L1**

PD-L1 tumor surface expression, which is positive in 40-100% in metastatic melanoma, has been studied intensively as a potential biomarker for the selection of patients that qualify for anti-PD-1 therapy, but not without any obstacles. In early clinical trials, PD-L1 expression has been associated with response to PD1/PD-L1 inhibition (19-21). Later trials reported a response to PD1/PD-L1 checkpoint inhibitors in up to 47% of PD-L1-negative melanomas (22). Also, the potentially heterogeneous expression and fast dynamics of PD-L1 expression make clinical implementation

difficult (19). By labeling of a PD-L1 binding precursor with Fluoride-18 ( $^{18}\text{F}$ ), serial [ $^{18}\text{F}$ ]PD-L1-PET scanning can be used to assess whole body distribution, pharmacokinetics (PK) and pharmacodynamics (PD) and to relate findings to treatment effects. This could help in patient selection and possibly serve as an (early) biomarker for response to PD1/PD-L1 checkpoint inhibitors in the future.

In NSCLC PD-L1 expression is associated with response to therapy, as illustrated above. Therefore, identification of tumors with high PD-L1 expression in this patient category can be of high clinical value.

## **2.4 Rationale**

Clinical trials have shown efficacy of PD1/PD-L1 checkpoint inhibitors in multiple solid tumors, including melanoma and NSCLC. Whole body information with regard to target presence, drug kinetics and dynamics, as well as binding of PD-L1 targeting agents to the immune system cells is lacking. Molecular imaging of PD-L1 could lead to new insights on heterogeneity of PD-L1 expression in metastatic lesions and be of help in the prediction of response to PD1/PD-L1 inhibitors in a non-invasive manner.

## **3. OBJECTIVES**

### **3.1 Phase one**

Primary Objective:

- To determine the optimal timing of imaging for [ $^{18}\text{F}$ ]PD-L1 tracer imaging of inoperable melanoma and NSCLC.

### **3.2 Phase two**

Primary Objective:

- To assess the association of PD-L1 expression as measured by PD-L1 tracer uptake on PET and PD-L1 expression as measured by immunohistochemical (IHC) staining for PD-L1 of corresponding tumor lesions.
- To correlate response to anti-PD1 treatment with differences in tumor PD-L1 and PD1 expression between baseline and after 6 weeks of treatment, as quantified by PET and IHC analysis.

Secondary objectives:

- To characterize between-subject and within-subject variability in accumulation of the [ $^{18}\text{F}$ ]PD-L1 tracer in melanoma and NSCLC metastases.

#### 4. STUDY DESIGN

This is a single center feasibility study for the application of [ $^{18}\text{F}$ ]PD-L1 PET in patients with metastatic melanoma and NSCLC treated with anti-PD-1 therapy. The study will consist of a pharmacokinetics phase (phase one) and tracer validation phase (phase two). Phase one will be performed in a maximum of 5 patients. A [ $^{18}\text{F}$ ]PD-L1 PET scan will be performed at baseline and six weeks after treatment initiation. Upon finishing phase one, the optimal time for tracer injection will be determined prior to the start of phase two. In phase two a [ $^{18}\text{F}$ ]PD-L1 PET-CT scan will be performed in 10 patients at baseline and six weeks after treatment initiation. Also, a biopsy will be taken from at least one accessible tumor location after the PET-scan at baseline and the PET-scan after six weeks of therapy.

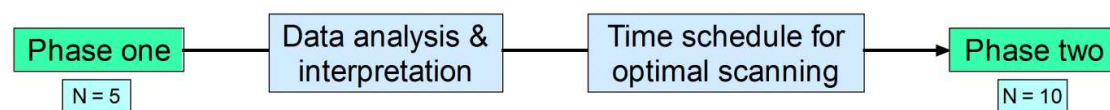


Fig.1 – General study overview.

#### 5. STUDY POPULATION

##### 5.1 Study population

All patients should be diagnosed with stage IV metastatic melanoma or non-small cell lung cancer. Patients should be eligible for treatment with nivolumab. Patients participating in other trials with this agent may participate in phase one of this study, as long as this does not interfere with either one of the study protocols. Patients participating in a clinical trial for nivolumab (e.g.: An open-label, single-arm, phase II, multicenter study to evaluate the efficacy of nivolumab in metastatic melanoma patients with symptomatic brain metastases) may participate in phase two of this study as well.

The University Medical Center Groningen (UMCG) is a referral center for metastatic melanoma. Yearly, approximately 100 patients start systemic treatment for metastatic melanoma in this study center, of which 30 are eligible for nivolumab therapy each year. Out of this group approximately 1-2 patients a month will be recruited for inclusion in this study.

## 5.2 Inclusion criteria

In order to participate in this study, a subject must meet all of the following eligibility criteria:

1. Subjects must sign informed consent prior to inclusion in this trial.
2. Subjects must be  $\geq 18$  years of age and competent to give informed consent.
3. Subjects must be diagnosed with histologically confirmed stage IV melanoma or non-small cell lung cancer.
4. At least one radiologic new lesion in the brain by MRI, which should be measurable by RANO-BM criteria (longest diameter  $\geq 10$  mm and perpendicular diameter  $\geq 5$  mm). Lesions with prior local treatment (*i.e.*, SRT or surgical resection) can be considered measurable if there has been demonstrated progression since the time of local treatment. Leptomeningeal involvement is allowed, but cannot be used as target lesion.
5. At least one easy accessible metastatic melanoma lesion of which a biopsy can be taken.
6. Subjects must be treatment-naïve to nivolumab. (also as adjuvant treatment)
7. Subjects must score at least 1 or higher on the Eastern Cooperative Oncology Group (ECOG) Performance Status.(25)
8. Subjects must have adequate organ function as defined by the following laboratory values (determined within 28 days prior to randomization/registration):
  1. White blood cells (WBC)  $\geq 2000$  / $\mu$ L
  2. Absolute neutrophil count (ANC)  $\geq 1500$  / $\mu$ L
  3. Platelets  $\geq 100 \times 10^3$  / $\mu$ L
  4. Hemoglobin  $\geq 9$  g/dL or  $\geq 5.6$  mmol/L
  5. Serum creatinine  $\leq 1.5$  times upper limit of normal (ULN) or creatinine clearance  $> 40$  ml/min (using the Cockcroft-Gault formula)
  6. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT)  $\leq 3$  times ULN
  7. Bilirubin  $\leq 1.5$  times ULN (Except patients with the Gilbert Syndrome, for whom a maximum of  $\leq 3.0$  mg/dL is acceptable)
9. Women of childbearing potential (WOCBP) should have a negative urine or serum pregnancy test within 7 days prior to receiving the first administration of nivolumab. Women with non-childbearing potential may be included if they are either surgically sterile or have been postmenopausal for  $\geq 1$  year.
10. WOCBP and men who are sexually active with WOCBP must agree to use appropriate method(s) of contraception. (see section 5.2)

### 5.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

1. Prior treatment with an anti-PD-1, anti-PD-L1, anti-PD-L2 antibody, or any other antibody or drug specifically targeting T-cell costimulation or immune checkpoint pathways, except anti-CTLA4 antibody.
2. Subjects who have not recovered to Common Terminology Criteria for Adverse Events (CTCAE) v4.0 Grade 1 or better from the adverse events due to previous cancer therapy.
3. Evidence for an active, known or suspected autoimmune disease. Subjects diagnosed with vitiligo, type I diabetes mellitus, residual hypothyroidism due to autoimmune condition only requiring hormone replacement, psoriasis not requiring systemic treatment, or conditions not expected to recur in the absence of an external trigger are permitted to enroll.
4. Treatment with corticosteroids in an increasing dosage in the 7 days prior to the first administration of nivolumab. (A stable or decreasing dosage of  $\leq 4$  mg dexamethasone or equivalent is allowed. In addition, inhaled or topical steroids and adrenal replacement doses are permitted in the absence of active autoimmune disease.)
5. Previous malignancies (except non-melanoma skin cancers, in situ bladder cancer, gastric or colon cancers, cervical cancers/dysplasia or breast carcinoma in situ) unless a complete remission was achieved at least 1 years prior to study entry and no additional therapy is required or anticipated to be required during the study period.
6. A severe hypersensitivity reaction to prior treatment with a monoclonal antibody, or known hypersensitivity to study drugs components.
7. A positive test for hepatitis B virus surface antigen (HBV sAg) or hepatitis C virus ribonucleic acid (HCV antibody) indicating acute or chronic infection.
8. Known history of testing positive for human immunodeficiency virus (HIV) or known acquired immunodeficiency syndrome (AIDS).
9. Any serious or uncontrolled medical disorder or active infection that, in the opinion of the investigator, may increase the risk associated with study participation, study drug administration, or would impair the ability of the patients to receive protocol therapy.
10. A known psychiatric or substance abuse disorder that could interfere with cancer therapy.
11. Women of childbearing potential with a positive serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) within 24 hours prior to the start of nivolumab.
12. Breastfeeding women.
13. Inability to comply with other requirements of the protocol.



#### 5.4 Sample size calculation

The total sample size of the study is 15 patients at maximum. A maximum of 5 patients will participate in phase one and 10 patients will participate in phase two. With an expected accrual rate of 1-2 patients per month, the intervention part of the study could be completed within 12 months.

### 6. TREATMENT OF SUBJECTS

#### 6.1 Investigational product/treatment

The intervention of this study is a [ $^{18}\text{F}$ ]PD-L1 PET scan. The radiolabeled PET tracer [ $^{18}\text{F}$ ]PD-L1 is developed by Bristol-Myers Squibb and will be produced in our center according to GMP guidelines. For additional data about the precursor material and preclinical data of the tracer we refer to the investigators brochure, version 1, 14 Dec 2015.

#### 6.2 Use of co-intervention

##### *Corticosteroids*

The use of systemic corticosteroids at baseline and during nivolumab therapy, should be avoided because of their potential interference with the pharmacodynamic activity and efficacy of nivolumab. A stable or decreasing dosage of  $\leq 4$  mg dexamethasone or equivalent is allowed. In addition, inhaled or topical steroids and adrenal replacement doses  $> 4$  mg daily dexamethasone equivalents in the absence of active autoimmune disease are permitted.

##### *Contraception methods*

WOCBP and men with reproductive potential must be willing to practice acceptable methods of birth control during the study. "WOCBP" is defined as any female who has experienced menarche and who has not undergone surgical sterilization (hysterectomy or bilateral oophorectomy) or who is not postmenopausal. Menopause is defined clinically as 12 months of amenorrhea in a woman over 45 in the absence of other biological or physiological causes. In addition, women under the age of 55 must have a documented serum follicle stimulating hormone (FSH) level greater than 40 mIU/mL.

WOCBP should use an adequate method to avoid pregnancy for 23 weeks (30 days plus the time required for nivolumab to undergo five half-lives) after the last dose of investigational drug. Men who are sexually active with WOCBP must use any contraceptive method with a failure rate of less than 1% per year. Men receiving nivolumab and who are sexually active with WOCBP will be instructed to adhere to contraception for a period of 31 weeks after the last dose of investigational product.

## 7. INVESTIGATIONAL PRODUCT

See concept IMPD attached for PET radiotracer [ $^{18}\text{F}$ ]PD-L1, version 2.0, date 17-05-2016.

**8. NON-INVESTIGATIONAL PRODUCT****8.1 Name and description of non-investigational product(s)**

See section 3.1, page 20, of the investigators brochure for nivolumab.

**8.2 Summary of findings from non-clinical studies**

See section 4, page 21-29, of the investigators brochure for nivolumab.

**8.3 Summary of findings from clinical studies**

See section 5, page 29-88, of the investigators brochure for nivolumab.

**8.4 Summary of known and potential risks and benefits**

For efficacy and safety results of previous clinical studies, see section 5.4 and 5.5 of the investigators brochure for nivolumab.

**8.5 Description and justification of route of administration and dosage**

See section 3.2.1, page 20, of the investigators brochure for nivolumab.

**8.6 Dosages, dosage modifications and method of administration**

See section 5.2 of this clinical trial protocol for a description of dose calculation and modification for nivolumab.

**8.7 Preparation and labelling of Non Investigational Medicinal Product**

See section 3.2.2., page 20-21, of the investigators brochure for nivolumab.

**8.8 Drug accountability**

See section 3.2.3., page 21, of the investigators brochure for nivolumab for recommended storage and use conditions.

## 9. METHODS

### 9.1 Primary study parameter/endpoint

Phase one concerns the pharmacokinetic sub-study to assess the dynamics and kinetics of the PD-L1 tracer as measured by PET imaging in patients with inoperable melanoma treated with a PD1 immune checkpoint inhibitor (nivolumab). For assessment of the tracer pharmacokinetics and optimum time for scanning, serial whole body PET scans will be acquired at baseline and six weeks after treatment initiation in these patients in order to determine the tracer kinetics in major organs.

Phase two will assess the association between PD-L1 expression on PET scan and PD-L1 expression on IHC.

For detailed information on study procedures, including scan sequences, we refer to paragraph 8.6 of this protocol.

### 9.2 Secondary study parameters/endpoints

Heterogeneity of tracer uptake between separate tumor lesions will be determined both for separate lesions within one patient (intra-patient heterogeneity) and for lesions in different patients (inter-patient heterogeneity).

Differences in PD-L1 expression, both assessed by PET scanning and IHC, between baseline and after 6 weeks of treatment with a PD1 immune checkpoint inhibitor will be correlated to response to treatment as measured by standard of care radiological response assessment.

### 9.3 Randomisation, blinding and treatment allocation

This trial is a single-arm study. Therefore no randomisation or blinding will take place. A maximum of 5 patients will be enrolled in phase one of this study. Thereafter, another 10 patients will be enrolled in phase two.

### 9.4 Study procedures

#### 9.4.1 Patient evaluation

Written informed consent will be obtained from patients before study entry. Patients will be screened for meeting all eligibility criteria. At study entry the following data are collected: medical history, characteristics of the primary tumor and metastases, location of a tumor lesion that could be biopsied, co-medication, contraceptive use, pregnancy, ECOG performance score, and a physical examination including vital signs, weight, length and blood pressure. To exclude pregnant women from the study, all WOCBP must undergo a urine pregnancy test (beta hCG) before study entry. To

exclude patients with an acute or chronic hepatitis infection, HBV sAg and HCV antibody will be determined. In addition, blood tests are acquired (e.g. complete blood count and blood chemistry), which are part of the regular treatment with nivolumab.

#### 9.4.2 PET procedure and quantification of tracer uptake

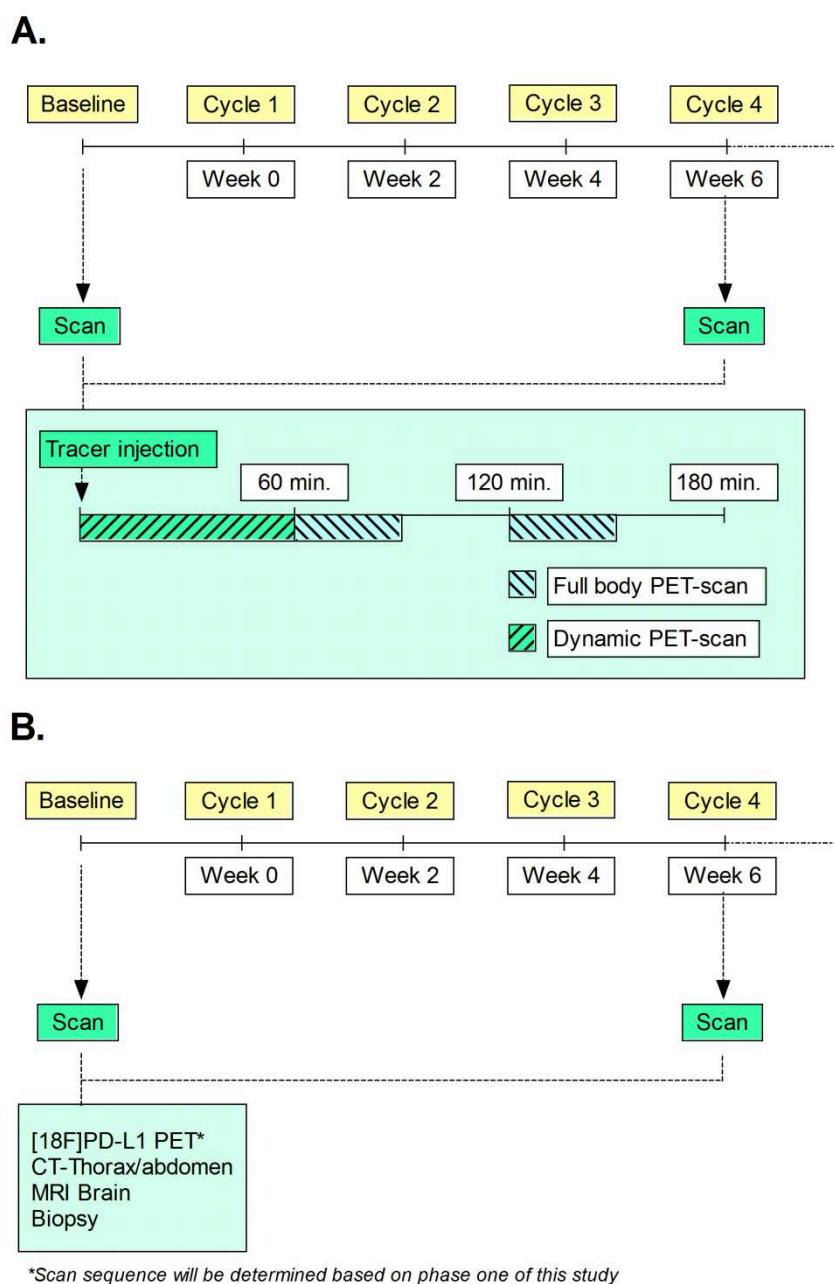
In phase one, the tracer kinetics will be measured in order to determine the optimal tracer distribution time and to evaluate which simplified method is best suited for the quantitative analysis of the tracer uptake. For this purpose, 5 patients will undergo an extended PET acquisition protocol, both at baseline and after 6 weeks of treatment with immunotherapy. Immediately after injection of the tracer a dynamic PET scan will be acquired of the chest (single bed position; 60 minutes). The dynamic PET scan will be followed by two total body PET scans (head to toe; 12 bed positions, 3 minutes per bed position). The first total body PET starts at 60 minutes after tracer injection (i.e. immediately after the dynamic PET scan). After this scan (ca. 90-100 minutes after tracer injection), the patient can shortly leave the PET camera. An additional total body scan will be performed at 120 minutes after tracer injection. The two total body scans will be used to quantify tracer uptake in tumors and tumor contrast throughout the body in order to establish the optimal time point for imaging. If during this phase of the study either one of the two total body PET scans seems obsolete due to low tracer availability, small differences between both scans or whatever other reason, one scan will be omitted to minimize burden in the following patients.

In phase two, a single PET-scan will be performed at baseline and after six weeks of treatment at the optimal time point after tracer injection as determined in phase one. Both scans will be accompanied by a low-dose or diagnostic CT-scan for anatomic reference purposes. The scans will be performed one hour or two hours after tracer injection, depending on the optimal time of tracer injection as established in phase I.

Before the start of the imaging procedure, a venous catheter is placed in one arm and patients will be injected with approximately 200 MBq (0,63mL) [<sup>18</sup>F]PD-L1. Patients will be asked to drink 1 liter of water to promote the renal clearance of unbound tracer from the circulation in order to enhance signal to background ratio. Prior to scanning, patients will be asked to go to the toilet to empty their bladder in order to reduce the radiation burden to the patient and prevent high bladder uptake on the PET scan.

Vital signs (blood pressure and heart rate) will be measured before, 10 minutes after the injection of [<sup>18</sup>F]PD-L1 and directly after the PET-CT scan. Blood samples will be taken before and after each PET-CT scan to analyze complete blood count and blood chemistry.

During the PET-scan, multiple venous samples will be taken to assess tracer metabolism.



*Fig.2 – A: Detailed overview of the procedures in phase one of the study. Patients will be scanned both at baseline and after six weeks of treatment during a two hour scan protocol. B: Detailed overview of the procedures in phase two of the study. Again, patients will be scanned both at baseline and after six weeks of therapy. At both time points, only a single PET-scan will be performed. To assess PD-L1 expression by IHC a biopsy will be taken after both PET-scans.*

For quantification of tracer uptake, the investigator visually identifies tumor lesions on a PET or CT scan and determines a region-of-interest (ROI) around these lesions. [ $^{18}\text{F}$ ]PD-L1-uptake in these ROI's will be corrected for body weight and injected dose and quantified as standardized uptake value

(SUV<sub>max</sub> and SUV<sub>mean</sub>). The absolute tracer uptake will be assessed both at baseline and six weeks after treatment initiation.

#### 9.4.3 CT scan

During phase two, each PET scan will be accompanied by a diagnostic CT scan. The CT scans performed at baseline of treatment with anti-PD1 treatment are part of care as usual. If a diagnostic CT scan has been performed shortly before to inclusion ( $\leq 2$  weeks) this will not be repeated. CT imaging consists of examination of chest, abdomen and pelvis. Response to treatment of tumor lesions will be evaluated according to the RECIST 1.1 criteria.

#### 9.4.4 MRI scan

During phase two, each PET scan will also be accompanied by a MRI scan of the brain. These MRI scans are also part of regular care at baseline of treatment with anti-PD1 treatment. If a recent brain MRI scan has been performed prior to inclusion ( $\leq 2$  weeks) this will not be repeated at baseline. Before the MRI scan will be performed, gadolinium will be administrated intravenously which acts as a contrast agent. In subjects with a contraindication for an MRI scan, this could be replaced by a contrast-enhanced head CT scan.

#### 9.4.5 Biopsy

In phase two of the study at least one biopsy sample will be collected from an accessible tumor lesion after each PET scan. If possible, these tissue samples will be obtained from the same tumor lesions at baseline and after 6 weeks of treatment. Ideally biopsies will be taken from both [18F]PD-L1 PET positive as well as negative lesions within the same patient. Determination of the biopsy site will be based on tumor localization on previous diagnostic imaging studies and will be performed according to current safety criteria. A biopsy will be performed under local anesthetics and ultrasonographic or CT-guidance. Biopsy material will be FFPE.

#### 9.4.6 Immunohistochemistry

Immunohistochemistry will be performed on FFPE tumor blocks mounted on 3-aminopropyltriethoxysilane-coated glass slides. To determine PD-L1 and PD1 expression on melanoma cells and tumor infiltrating immune cells respectively, these slides will be stained with anti-PD-L1 and anti-PD1 monoclonal antibodies. For quantitative scoring of positively stained cells,

the percentage of positive cells, the intensity of staining and the pattern of staining will be accounted for.

### **9.5 Withdrawal of individual subjects**

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

### **9.6 Replacement of individual subjects after withdrawal**

Subjects that withdraw from the study will be replaced in order to achieve the number of patients needed for the study endpoints; see 'sample size calculation'.

### **9.7 Premature termination of the study**

Premature termination will only be applied when serious unforeseen adverse events are detected which could be clearly attributed to the investigational product, and which prompt a premature termination of the study.



## 10. SAFETY REPORTING

### 10.1 Section 10 WMO event

In accordance to section 10, subsection 1, of the WMO, the investigator will inform the subjects and the reviewing accredited METC if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited METC, except insofar as suspension would jeopardise the subjects' health. The investigator will take care that all subjects are kept informed.

### 10.2 AEs, SAEs and SUSARs

#### 10.2.1 Adverse events (AEs)

AEs are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the investigational product. All AEs reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

#### 10.2.2 Nonserious adverse events

A nonserious adverse event is an AE not classified as serious. The collection of nonserious AE information should begin at initiation of study drug. All nonserious AEs (not only those deemed to be treatment-related) should be collected continuously during the treatment period and for a minimum of 100 days following the last dose of study treatment.

Nonserious AEs should be followed to resolution or stabilization, or reported as SAEs if they become serious. Follow-up is also required for nonserious AEs that cause interruption or discontinuation of study drug and for those present at the end of study treatment as appropriate.

#### 10.2.3 Serious adverse events (SAEs)

All SAEs reported spontaneously by the subject or observed by the investigator or his staff will be recorded according to CTCAE v4.0. A SAE is any experience that suggests a significant hazard, contraindication, side effect or precaution. It is any AE that at any dose fulfills at least one of the following criteria:

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

- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect;
- is a potential drug induced liver injury (DILI);
- is a suspected transmission of an infectious agent (eg, pathogenic or nonpathogenic) via the study drug;
- is a pregnancy, overdose, or secondary cancer;
- Any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered a serious adverse experience when, based upon appropriate medical judgement, the event may jeopardize the subject or may require an intervention to prevent one of the outcomes listed above.

Medical and scientific judgment should be exercised in deciding whether expedited reporting to the sponsor is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or requires intervention to prevent one of the outcomes listed in the definitions above. These situations should usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse. An unexpected Adverse Event is one, the nature or severity of which is not consistent with the applicable product information.

The causal relationship to study drug is determined by a physician and should be used to assess all adverse events (AE). The causal relationship can be one of the following:

- Related: There is a reasonable causal relationship between study drug administration and the AE.
- Not related: There is not a reasonable causal relationship between study drug administration and the AE.

The term "reasonable causal relationship" means there is evidence to suggest a causal relationship.

The term severe is a measure of intensity, thus a severe adverse event is not necessarily serious. For example, nausea of several hours' duration may be rated as severe, but may not be clinically serious. Following the subject's written consent to participate in the study, all SAEs, whether related or not related to study drug, must be collected, including those thought to be associated with protocol-specified procedures. All SAEs must be collected that occur within 100 days of discontinuation of dosing.

In addition, a SAE that occurs after this time, if considered related to test "drug", should be reported. Such preliminary reports will be followed by detailed descriptions later which will include copies of hospital case reports, autopsy reports and other documents when requested and applicable. For

SAEs, the following must be assessed and recorded on the adverse events page of the Case Report Form: intensity, relationship to test substance, action taken, and outcome to date.

The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 15 days after the sponsor has first knowledge of the serious adverse reactions.

SAEs that result in death or are life threatening should be reported expedited. The expedited reporting will occur not later than 7 days after the responsible investigator has first knowledge of the adverse reaction. This is for a preliminary report with another 8 days for completion of the report.

Besides reporting to the accredited METC, all SAE's that occur following the subject's written consent to participate in the study through 100 days of discontinuation of dosing must be reported to the sponsor BMS Worldwide Safety. (email: [Worldwide.Safety@BMS.com](mailto:Worldwide.Safety@BMS.com); fax: 609-818-3804)

All SAEs must be collected that occur during the screening period. If applicable, SAEs must be collected that relate to any protocol-specified procedure (eg, a follow-up skin biopsy). The investigator should report any SAE that occurs after these time periods that is believed to be related to study drug or protocol-specified procedure.

SAEs, whether related or not related to study drug, and pregnancies must be reported to BMS within 24 hours. SAEs must be recorded on BMS or an approved form; pregnancies on a Pregnancy Surveillance Form.

If only limited information is initially available, follow-up reports are required. (Note: Follow-up SAE reports should include the same investigator term(s) initially reported.)

If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, a follow-up SAE report should be sent within 24 hours to the BMS (or designee) using the same procedure used for transmitting the initial SAE report.

All SAEs should be followed to resolution or stabilization.

#### 10.2.4 Suspected unexpected serious adverse reactions (SUSARs)

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious (see chapter 9.2.2);

2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:
  - Summary of Product Characteristics (SPC) for an authorised medicinal product;
  - Investigator's Brochure (IB) for an unauthorised medicinal product.

The sponsor will report expedited the following SUSARs through the web portal *ToetsingOnline* to the METC:

- SUSARs that have arisen in the clinical trial that was assessed by the METC;
- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern.

The expedited reporting of SUSARs through the web portal *ToetsingOnline* is sufficient as notification to the competent authority.

The sponsor will report expedited all SUSARs to the competent authorities in other Member States, according to the requirements of the Member States.

The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

The principal investigator (PI) is responsible for reporting SUSAR's. All coordinating investigators of subcenters should inform the principal investigator as soon as possible. All SUSAR's are reported unblinded to the competent authorities.

### **10.3 Annual safety report**

In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, and competent authorities of the concerned Member States.

This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

### **10.4 Follow-up of adverse events**

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

SAEs need to be reported till end of study within the Netherlands, as defined in the protocol

## 11. STATISTICAL ANALYSIS

This is an exploratory, feasibility study that serves to provide base case statistics for further prospective research. Primarily descriptive statistics will be used. Demographic and baseline characteristics will be summarized using means, standard deviations, medians, and ranges for continuous variables, and proportions for categorical variables. The PET results will be both qualitatively and quantitatively assessed in 15 patients. Categorical data will be assessed using the Fisher's exact test. Continual data (paired and unpaired) will be assessed using non-parametrical tests (i.e. Mann-Whitney U test and Kruskal-Wallis test).

### 11.1 Primary study parameter(s)

Tracer pharmacokinetics and the optimal time for scanning will be determined from the data obtained by the serial PET scans in first 5 patients. Output parameters are the radiation absorbed dose to major organs, the effective dose equivalent for patients, the recommended maximum injected dose for [ $^{18}\text{F}$ ]PD-L1 and the optimal time point for scanning after tracer administration.

PD-L1 expression in melanoma tumor lesions will be quantified by measuring tracer uptake in tumor lesions. Tracer uptake will be corrected for body weight and injected dose and quantitatively assessed as standardised uptake value ( $\text{SUV}_{\text{mean}}$  with associated standard deviation and  $\text{SUV}_{\text{max}}$ ), which is calculated using the formula:  $[\text{tissue activity concentration (MBq/g)}]/[(\text{injected dose (MBq)}/\text{body weight (g)})]$ . SUV will be calculated for all predefined ROI's on the PET-CT scan at baseline. On the PET-CT scan at week 6 of treatment, SUV will be determined for all lesions detected at baseline as well as all newly detected tumor lesions.

Tumor uptake of [ $^{18}\text{F}$ ]PD-L1 will be correlated with the amount of PD-L1 expression of melanoma cells and PD-1 and PD-L1 expression on tumor infiltrating immune cells in melanoma lesions. PD-L1 and PD1 expression will be measured by immunohistochemistry. Results of immunohistochemical staining will be described as a semi-quantitative score using the percentage of positive cells (continuous variable), intensity and pattern of staining (discrete variable). This semi-quantitative score will then be correlated to SUV of the corresponding lesion on the [ $^{18}\text{F}$ ]PD-L1 PET. Correlation will be expressed as a Pearson/Spearman correlation coefficient depending on distribution of data. Results will be displayed in graphs.

### 11.2 Secondary study parameter(s)

Heterogeneity between tracer uptake of separate tumor lesions will be determined. For this purpose the absolute difference in SUV between the two PET scans will be determined for all separate lesions and will be visualised in a table. Heterogeneity of this absolute difference in SUV will be analysed for

lesions in one patient (intra-patient heterogeneity) and for lesions between patients (inter-patient heterogeneity).

Although the study population is too small to generate statistical relevant information, the ability of PD-L1 expression to predict response to anti-PD1 treatment will be assessed as a secondary parameter. For this purpose the different lesions identified on the first PET-CT scan, will be qualitatively scored as responding (stable disease, partial or complete regression) or non-responding (progressive disease) according to the RECIST 1.1 criteria. The measured response will then be correlated to the absolute increase in SUV between the two PET scans for PD-L1 expression on PET and response will be correlated to the difference in immunohistochemical score for PD-L1 expression in tumor tissue samples.

## **12. ETHICAL CONSIDERATIONS**

### **12.1 Regulation statement**

The study will be conducted according to the principles of the Declaration of Helsinki (Amended by the 64th WMA General Assembly, Fortaleza, Brazil, October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO) and other guidelines, regulations and Acts.

### **12.2 Recruitment and consent**

Treating physicians can refer a patient to the investigator (medical doctor) for participation in the study. Information about the study will appear on the website of the medical oncology department ([www.medischeoncologiegroningen.nl](http://www.medischeoncologiegroningen.nl)).

Patients will be informed by one of the investigators (medical doctor) and under the supervision of the principle investigator. Patients will be informed orally during a patient visit in the UMCG and will receive a patient information letter (attached as a separate document). Patients will have a minimum of 1 week to consider their decision. When a patient wants to participate a written informed consent form has to be signed (attached as a separate document). After completion of the study procedures the patient will be referred back to the treating physician.

### **12.3 Objection by minors or incapacitated subjects (if applicable)**

All subjects will be  $\geq 18$  years and competent.

### **12.4 Benefits and risks assessment, group relatedness**

Molecular imaging with a PET scan is an upcoming technique for obtaining non-invasive whole body information about specific molecular targets. In this imaging study we want to visualize PD-L1 expression in tumor lesions of patients with metastatic melanoma. This tumoral PD-L1 expression could serve as a predictor of response to anti-PD1 treatment. Results of this study could provide valuable information about the whole body distribution of PD-L1 in tumor lesions and heterogeneity in this distribution. In addition, this study will explore how intratumoral PD-L1 and PD1 expression is influenced by anti-PD1 treatment.

Patients will not benefit from participation in this study directly, but their participation helps to develop a new imaging technique which could provide more insights in PD-L1 expression in tumor lesions and predictive value of this PD-L1 expression.



Patients will be treated with anti-PD1 as part of their regular treatment or in the context of a clinical trial. So, this treatment will not cause any additional risk or burden to the patient in this imaging study. Patients will also get regular follow-up to assess disease control.

The tracer was well tolerated in pre-clinical toxicology studies performed in cynomolgus monkeys. For detailed information, we refer to the IB, version 1, 14 Dec 2015.

The expected applied injected activity of [ $^{18}\text{F}$ ]PD-L1 is 200 MBq, as is the case for most other  $^{18}\text{F}$ -labeled tracers. This dose will be used in phase one of the study and could be changed according to the pharmacokinetics results for patients in phase two of the study. The radiation burden for a dose of 200 MBq is estimated to be comparable to that of other  $^{18}\text{F}$ -labeled PET tracers (approximately 4.4 mSv). Patients will receive 2 serial PET scans each accompanied by a low dose CT scan which adds up to a total dose of 11.8 mSv. The radiation burden of these patients complies with category III (moderate risk) according to the International Commission on Radiological Protection (ICRP) guidelines (ICRP publication 62). According to the investigators, the radiation burden in this category of patients is justified by the information that can be obtained from this study, previously described.

Biopsies are mandatory in phase two of this study. This is an invasive procedure. Preferably biopsies will be taken from easily accessible lesions to minimize the burden to the patient. Patients will be informed explicitly about potential safety issues. Although biopsies are generally considered to be safe, it could be a painful intervention and carries the risk of bleeding at the puncture site.

Whenever possible, all procedures that are part of the study protocol will be planned on a day that the patient already has a regular visit to the hospital as part of their treatment.

### **12.5 Compensation for injury**

The sponsor/investigator has a liability insurance which is in accordance with article 7, subsection 6 of the WMO.

The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO and the Measure regarding Compulsory Insurance for Clinical Research in Humans of 23th June 2003). This insurance provides cover for damage to research subjects through injury or death caused by the study.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

### **12.6 Incentives (if applicable)**

For each day of patient related study procedures, the subjects will receive compensation for travelling expenses (€ 0.19/km) and a ticket for free parking.

### **13. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION**

#### **13.1 Handling and storage of data and documents**

As a result of the regulations for radiation exposure/ safety, the information that is obtained at the Department of Nuclear Medicine and Molecular Imaging will not be stored anonymously. However, personal information will only be accessible for medical doctors affiliated to that department and handled confidentially. Histological material that is obtained during the study is regarded as routine clinical information and kept for a longer period according to standard protocols. This material is accessible for pathologists.

For other data management, study subjects will receive a code. The code will consist of the study ID number followed by patient number (1-15). The key to the code (number linked to patient) is safeguarded by the investigator. The study code assigned to the patients will also be used in the collection of all the study results by the investigator who will perform the data-analysis. An overview of all data and data-analysis is made according to this code, so that the final results cannot be traced back to the patients by another person than the investigators involved in the study (in compliance with the Dutch Personal Data Protection Act).

Data will be stored for a maximum period of 15 years after the study is finished.

#### **13.2 Monitoring and Quality Assurance**

On-site monitoring will take place conform the NFU (Nederlandse Federatie van Universitaire Medisch Centra)-guideline “Kwaliteitsborging van mensgebonden onderzoek 2010” by the appointed monitor. Monitoring will take place to assure the quality and validity of the research data. The monitor will perform source data verification on the research data by comparing the data entered into the CRF with the available source documentation and other available documents. Source documents are defined as the patient’s hospital medical records, clinician notes, laboratory print outs, digital and hard copies of imaging, memos, electronic data etc. The monitor will verify the following items (100%): Patient flow (inclusion speed and dropout rate); Informed consent forms (presence, dates, signatures); Trial Master File and Investigator Files (presence of all documents), in-/exclusion criteria (using source documents); SAEs / SUSARs (number, missed, reporting procedures); study product (administration, accountability). After each control the monitor will send a written report to the sponsor (including a summary; quality assessment; summary of findings, deviations and shortcomings; possible solutions to warrant compliance with the protocol; final conclusion).

### **13.3 Amendments**

A 'substantial amendment' is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the METC and to the competent authority.

Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

### **13.4 Annual progress report**

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/serious adverse reactions, other problems, and amendments.

### **13.5 End of study report**

The sponsor will notify the accredited METC and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient's last visit.

In case the study is ended prematurely, the sponsor will notify the accredited METC and the competent authority within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC and the Competent Authority.

### **13.6 Public disclosure and publication policy**

The authors have no conflict of interest, no financial interest and no arrangement or affiliation with any commercial organization that may have a direct or indirect interest in the outcome of the study.

## 14. STRUCTURED RISK ANALYSIS

### 14.1 Potential issues of concern

#### 14.1.1 Level of knowledge about mechanism of action

The mechanism of action of the [ $^{18}\text{F}$ ]PD-L1 PET tracer has been studied extensively in preclinical models. See section 4.1, page 13 – 23 of the IB for PD-L1 Adnectin PET Tracer (BMS-986192) for detailed information.

#### 14.1.2 Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism

No clinical studies have been conducted with the [ $^{18}\text{F}$ ]PD-L1 PET tracer. This is the first study assessing the use of the [ $^{18}\text{F}$ ]PD-L1 PET tracer in human beings. (IB: section 5, page 28.)

#### 14.1.3 Can the primary or secondary mechanism be induced in animals and/or in *ex-vivo* human cell material?

The feasibility of the [ $^{18}\text{F}$ ]PD-L1 PET tracer has been shown in both mouse xenograft and cynomolgus monkey studies, as well as in human tissue (non small cell lung cancer samples). For more information see section 4.1.1.1 and 4.1.1.2, page 13 – 17 of the IB for PD-L1 Adnectin PET Tracer (BMS-986192).

#### 14.1.4 Selectivity of the mechanism to target tissue in animals and/or human beings

Tissue binding studies have been performed in vivo in cynomolgus monkeys and mice xenograft models and in vitro using human tissue. The [ $^{18}\text{F}$ ]PD-L1 PET tracer is highly selective for both cynomolgus monkey and human forms of the PD-L1 extracellular domain, but not to the extracellular domain of murine PD-L1. The [ $^{18}\text{F}$ ]PD-L1 PET tracer has shown high uptake in kidney, L2987 xenograft, lung and bone. For more detailed information see section 4.1, page 13 – 23 of the IB for PD-L1 Adnectin PET Tracer (BMS-986192) for detailed information.

#### 14.1.5 Analysis of potential effect

The tracer was well tolerated in pre-clinical toxicology studies performed in cynomolgus monkeys. (IB: section 4.3.1, page 26 and 27.) Genotoxicity and reproductive and developmental toxicity were not assessed due to reasons listed in sections 4.3.2 and 4.3.3, page 27 and 28 of the IB for PD-L1 Adnectin PET Tracer (BMS-986192).

#### 14.1.6 Pharmacokinetic considerations

Results of pre-clinical pharmacokinetic studies can be found in section 4.2, page 24 and 25 of the IB for PD-L1 Adnectin PET Tracer (BMS-986192).

#### 14.1.7 Study population

Our study population consists of stage IV melanoma patients with symptomatic brain metastases. Study subjects are at least 18 years of age and must understand and sign informed consent prior to study inclusion. For detailed information on the study population concerned we refer to section 4.1 - 4.3, page 15 – 17 of this protocol.

#### 14.1.8 Interaction with other products

Pharmacokinetic drug interactions have not been evaluated. (IB: section 4.2.5, page 25.)

#### 14.1.9 Predictability of effect

Since the investigational product in this study is a diagnostic agent, without an expected pharmacological effect, no biomarkers are used to predict such an effect.

### 14.2 Synthesis

This is the first time the [<sup>18</sup>F]PD-L1 PET tracer is tested in a clinical study, therefore no toxicology and overall risk data is available on the use of this product in human beings. Pre-clinical testing in cynomolgus monkeys has shown no toxicologic findings, as stated before. This toxicity study has shown a favourable safety profile in monkeys with a high safety margin relative to the anticipated tracer dose used in the human population. There is no available information concerning overdose with the study tracer. There is no specific antidote available. Upon suspicion of overdose and/or adverse reactions based on symptoms and/or signs supportive medical management should be provided. For further information see section 4.3 through 4.5, page 25 – 28 of the IB for PD-L1 Adnectin PET Tracer (BMS-986192).

## 15. REFERENCES

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