Phase 2 Study Comparing Pembrolizumab with Intermittent/Short-term Dual MAPK Pathway Inhibition Plus Pembrolizumab in patients harboring the BRAFV600 mutation (IMPemBra)

Version 6

PROTOCOL TITLE

Phase 2 Study Comparing Pembrolizumab with Intermittent/Short-term Dual MAPK Pathway Inhibition Plus Pembrolizumab in patients harboring the BRAFV600 mutation (IMPemBra).

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Independent expert (s)	***Michel van den Heuvel***
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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	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)
AE	Adverse Event
CA	Competent Authority
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
CTLA-4	cytotoxic T-lymphocyte-associated protein 4
DSMB	Data Safety Monitoring Board
EU	European Union
EudraCT	European drug regulatory affairs Clinical Trials
GCP	Good Clinical Practice
IB	Investigator's Brochure
IC	Informed Consent
IHC	Immunehistochemistry
irAE	Immune related adverse event
irRC	Immune related response criteria
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
LDH	Lactate dehydrogenase
METC	Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)
MSD	Merck Sharpe & Dohme
PD-1	Programmed death receptor-1
(S)AE	(Serious) Adverse Event
SPC	Summary of Product Characteristics (in Dutch: officiële productinfomatie IB1-tekst)
Sponsor	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.
SUSAR	Suspected Unexpected Serious Adverse Reaction
Wbp	Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)
WMO	Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen

SUMMARY

Rationale:

Targeting the PD-1/PD-L1 immune checkpoint pathway by e.g. pembrolizumab (MK-3475, Keytruda) is currently one of the most promising immunotherapeutic approaches in late stage melanoma with a response rate of 34% and long-term responses [1, 2]. In contrast to immunotherapies that are characterized by (so far) lower response rates, but long-term benefit [3], targeted therapies (BRAF or MEK inhibitors), can induce high response rates, but only of short duration [4-6], even when combined [7, 8]. These contrasting response patterns have early led to the idea of combining immune- and targeted therapy. However, initial attempts of combining vemurafenib or dabrafenib + trametinib with ipilimumab failed due to toxicity ([9], and Puzanov et al., abstract #2511, ASCO 2014). Combination of continuous BRAF+MEK (dabarefenib+trametinib) inhibition with PD-L1 blockade (MEDI4736, durvalumab), however, is feasible, induces T cell infiltration at week 2, and seems to induce prolonged tumor control (Ribas et al., abstract # 3003, ASCO 2015). However, individual immunohistochemistry analyses indicate that beyond 4-6 weeks the T cell tumor infiltration decreases again upon BRAF or BRAF+MEK inhibition (Jennifer Wargo, Robert Atkins, and Georgina Long, personal communications). Similar results we have also observed in on treatment biopsies beyond 6 weeks of treatment with BRAF or BRAF+MEK inhibition. When testing such combinations in a mouse model of human melanoma [10, 11] we found that BRAF and to a stronger extents BRAF+MEK inhibition induces indeed T cell melanoma infiltration (already at day 7) and shifts the CD8/Treg ratio favorably to the CD8+ effecter T cells (Blank et al., manuscript in preparation).

Thus, we postulate that combined BRAF+MEK inhibition can synergize (by antigen retrieval and modulation of intratumoral immune infiltrates) with checkpoint inhibition, as tested in this trial with one of the standard treatment anti-PD-1 antibodies, namely pembrolizumab. Furthermore current data indicate that such combination is safe, but that the continuous application of BRAF+MEK, as tested so far (Ribas et al., abstract # 3003, ASCO 2015) might be counterproductive.

Therefore this trial wants to analyze in an explorative manner different schemes of dabrafenib+trametinib (short continuous or intermittent) added to pembrolizumab and compare them to pembrolizumab monotherapy.

Objectives:

Primary objectives:

To explore the safety, feasibility, and the immune-activating capacity of different schemes of continuous/intermittent dabrafenib+trametinib during treatment with pembrolizumab as compared to pembrolizumab monotherapy

Secondary objectives:

- To determine rates of response at week 6, 12, week 18, and best overall response.

- To determine progression-free survival starting from randomization.

- To determine long-term toxicities of intermittent dabrafenib + trametinib during treatment with pembrolizumab as compared to pembrolizumab monotherapy

Explorative objectives:

- To describe time to progression beginning from week 12 (cohorts 2-4).

- Describe changes of immune parameters within the tumor.

- To explore the value of the e-Nose as an early biomarker for prediction of response to treatment.

Study design:

This is a Phase 2 trial consisting of 24 BRAFV600 mutation positive melanoma patients receiving the combination of dabrafenib + trametinib + pembrolizumab in 3 different dosing schemes and 8 patients receiving pembrolizumab standard monotherapy (Figure 1). All patients start with pembrolizumab standard therapy for 6 weeks and will then be randomized to continue pembrolizumab monotherapy or to receive additional intermitted/short-term dabrafenib + trametinib. Stratification will be according to baseline LDH level.

Study population:

Irresectable stage III and stage IV BRAFV600E/K mutation positive melanoma patients, naïve for PD-1/PD-L1 or CTLA-4 targeting immunotherapy, more than 18 years old.

Intervention:

Stage III/IV BRAFV600E/K mutation positive melanoma patients, naïve for CTLA-4/PD-1/PD-L1 blockade, will start treatment with pembrolizumab 200mg q3wk (Figure 1). After 6 weeks pembrolizumab monotherapy, the patients will be randomized to continue pembrolizumab for up to 2 years (cohort 1) or to switch to one of the experimental cohorts receiving either two times intermittent dabrafenib + trametinib for 1 week (cohort 2), or for 2 weeks (cohort 3), or continuous short-term dabrafenib + trametinib for 6 weeks (cohort 4). All cohorts continue afterwards with pembrolizumab monotherapy for up to 2 years in case of confirmed clinical benefit at week 18. Each cohort will consist of 8 patients.

Lab testing (incl. PBMC, EDTA blood, and serum collection) will be performed during screening, at baseline, at the indicated time points until week 18, and subsequently every three months.

Tumor biopsies/archival materials are required at baseline, at week 6, at week 9 (only cohorts 2-4, as there is a high likelihood that at week 12 and later, after the targeted therapy, there are no lesions anymore), week 12 and at week 18.

CT scans will be required at baseline, week 6, week 12, week 18, and subsequently every 3 months up to 2 years.

Another PBMC, EDTA blood, serum collection, and tumor biopsies will be performed at the timepoint of progression.

Figure 1

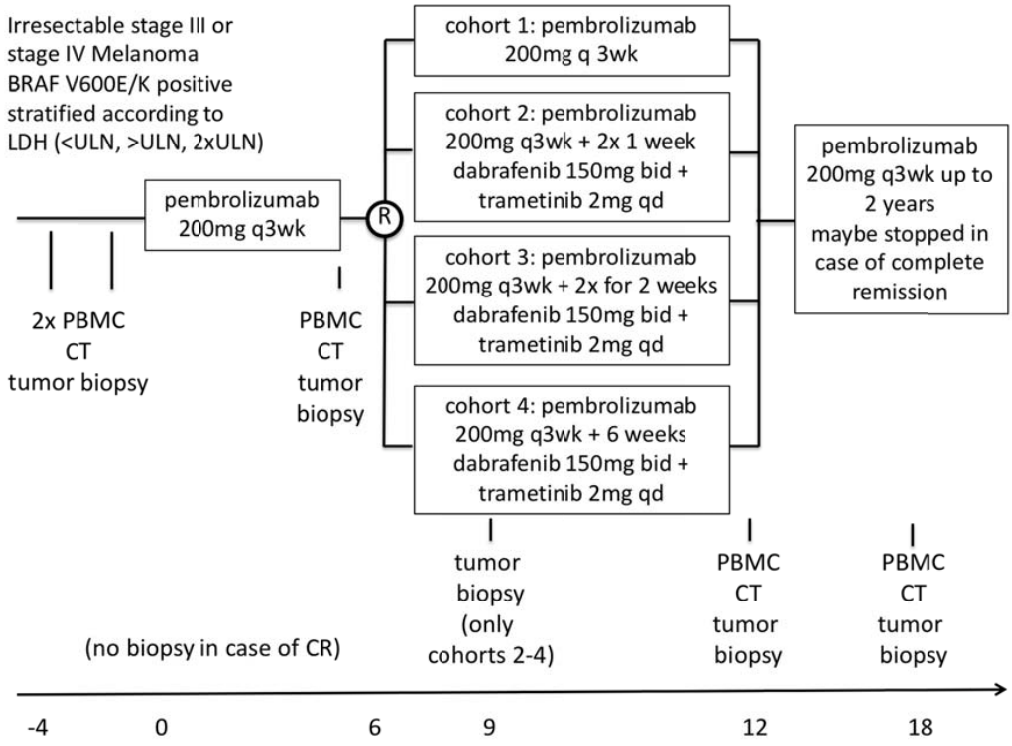


Figure 1: Schematic overview of IMPemBra study.

Main study parameters/endpoints:

Primary endpoints:

- Safety and feasibility as measured by SUSARs and adherence to the timelines of the study protocol (week 0 till week 18).

- Alterations in percentage of tumor infiltrating CD8+ T cells and in percentage of PD-1+CD8+ T cells in peripheral blood samples in the time interval pre-treatment to week 18 intrapatient, and interpatient, pembrolizumab only (cohort 1) versus pembrolizumab plus intermittent dabrafenib/ trametinib (cohorts 2-4).

Secondary endpoints:

- Rates of response at week 6, week 12, week 18, and best overall response, according to RECIST 1.1 criteria

- Progression-free survival (PFS) starting from randomization to progression using RECIST 1.1 criteria.

- Rate and type of late adverse events (beyond week 18, for up to 2 years)

Exploratory endpoints:

- As the short addition of dabrafenib+trametinib will induce for short time tumor regressions we will analyze cohorts 2-4 with a second baseline, namely the end of the targeted therapy at week 12, for progression free survival using to RECIST 1.1

- In addition to the primary readout (increase of PD-1+/CD8+ T cells), we will analyze the effect of the different therapy schemes on tumor immune cell infiltration (IHC for CD3, CD4, CD8, CD68, FoxP3, PD-L1, PD-L2, PD-1, CD11b, HLA) and on RNA signatures.

- The accuracy with which an eNose can discriminate between responders and non-responders at week 6 and 12, as compared to baseline.

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

Pembrolizumab monotherapy and the combination of dabrafenib and trametinib have both been tested as safe and effective treatment options in melanoma patients [2, 12], and have become approved standard therapies for late stage melanoma. The combination of dabrafenib+trametinib with anti-PD-L1 (MEDI4736, durvalumab) has been declared to be safe and induced increased tumor T cell infiltration (Ribas et al., abstract #3003, ASCO 2015). Nevertheless, adding an anti-PD-1 instead of anti-PD-L1 antibody could have another toxicity profile, despite their similarities in adverse events as monotherapies.

In contrast to Ribas et al. we will apply dabrafenib+trametinib for shorter time. This will expose the patients to less triple therapy, but might be more immunostimulatory, as we assume that continuous application reduces the initial tumor immune infiltration again. Thus a burden for the patients could be more frequent immune-related adverse events. Therefore we have included safety as primary readout into this study, despite the strong indication that the triple treatment is safe.

Additional burden for the patients participating in this trial (as compared to pembrolizumab monotherapy outside the study) are additional tumor biopsies that will be taken from easy accessible lesions (lymph node or subcutaneous lesions, which is an inclusion criterion), and more blood drawing.

1. INTRODUCTION AND RATIONALE

Background Melanoma

Melanoma is one of the fastest growing malignancies with more than 5,000 new diagnoses per year and almost 800 deaths in 2012 in the Netherlands [Dutch tumor registry (IKNL), http://www.iknl.nl], and approximately 130,000 new diagnoses worldwide, with 37,000 deaths (WHO, 2012). Surgical resection is the treatment of choice for localized melanoma and frequently results in cure for the early stages (stage I and II), with around 90% long-term survival rate for stage I melanoma [13]. However, patients with lymph-node involvement (stage III), including those detected only by sentinel node biopsy, have a high risk of local and distant relapse after surgery, and 5 year survival drops to 39-70% in this patient group [13]. No clinically significant improvement in survival has to date been achieved by adjuvant therapy in this patient group. Adjuvant radiotherapy has been shown to improve local disease control but no benefit in disease-free survival nor overall survival, has been achieved [14, 15]. High-dose interferon is currently the only systemic therapy for the adjuvant treatment of melanoma that is approved in some countries. However, questionable survival benefit (two out of three meta-analyses found no OS benefit [16-18]) and serious toxicity has led to the fact that interferon is not a generally accepted adjuvant treatment in Europe. Late stage melanoma (unresectable stage III and IV melanoma) was until a decade ago one of the most deadly malignancies [19]. With the advent of targeted and immunotherapies long-term survival has improved, but still less than 30% of patients survive beyond 3 years ([20], and Chapman et al., presentation SMR 2014).

Current standard treatment in late stage melanoma

With the growing understanding of genetic alterations in melanoma and immune modulation by T cell checkpoint molecules, two new therapeutic approaches have become standard therapy in late stage melanoma, both improving overall survival (OS) in randomized trials. Targeted therapy inhibiting the BRAFV600 mutation by selective BRAF inhibitors vemurafenib or dabrafenib significantly improved PFS and OS as compared to “standard” chemotherapy DTIC in randomized phase 3 trials [4, 5]. Combined MAPK pathway targeting by combination of selective BRAF and MEK inhibitors (dabrafenib+trametinib or vemurafenib+cobimetinib) has been shown to improve to improve OS further [8, 21], but long term tumor control seems to be rare, and only about 10% of BRAF inhibitor-treated patients remains progression-free at 4 years of continuous treatment (Chapman et al., presentation SMR 2014). No data are so far available for BRAF+MEK inhibitor combinations, due to too short follow up times.

T cell checkpoint blockade, e.g. antibody-mediated blockade of CTLA-4 and PD-1/PD-L1, has become another promising approach in treatment of late stage melanoma. Ipilimumab, a monoclonal antibody targeting CTLA-4, achieved improvement of OS in two randomized trials [22, 23]. While inducing less frequent responses than targeted therapies, long-term benefit of the responding patients is more common [20]. PD-1 blockade, by e.g. nivolumab or

pembrolizumab, induces even higher response rates at less toxicity than CTLA-4 blockade [1, 24], but the percentage of patients benefitting long-term from PD-1 blockade is not known yet due to the short follow up time of the phase 2/3 trials. Survival data from the phase 1 patients indicate 4-year survival rates of about 30-35% for PD-1 blockade [25], .

Background pembrolizumab and rationale for dose selection

The importance of intact immune surveillance in controlling outgrowth of neoplastic transformation has been known for decades. Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes (TILs) in cancer tissue, and favorable prognosis in various malignancies. In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells / FoxP3+ regulatory T-cells seems to correlate with improved prognosis and long-term survival in many solid tumors.

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene Pdcd1) is an Ig superfamily member, which has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD L2) (reviewed in [26]).

The mechanism by which PD-1 down-modulates T-cell responses is distinct from that of CTLA-4 [26]. PD-1 was shown to be expressed on activated lymphocytes including peripheral CD4+ and CD8+ T-cells, B-cells, T regs and Natural Killer cells [27]. Expression has also been shown during thymic development on CD4-CD8- (double negative) T-cells as well as subsets of macrophages and dendritic cells [27, 28]. The ligands for PD-1 (PD-L1 and PD-L2) are constitutively expressed or can be induced in a variety of cell types, including non-hematopoietic tissues as well as in various tumors [27]. Binding of either PD-1 ligand to PD-1 inhibits T-cell activation triggered through the T-cell receptor. PD-L1 is expressed at low levels on various non-hematopoietic tissues, most notably on vascular endothelium, whereas PD-L2 protein is only detectably expressed on antigen-presenting cells found in lymphoid tissue or chronic inflammatory environments. PD-L2 is thought to control immune T-cell activation in lymphoid organs, whereas PD-L1 serves to dampen unwarranted T-cell function in peripheral tissues. Although healthy organs express little (if any) PD-L1, a variety of cancers were demonstrated to express abundant levels of this T-cell inhibitor. PD-1 has been suggested to regulate tumor-specific T-cell expansion in subjects with melanoma (MEL) [29]. This suggested that the PD-1/PD-L1 pathway plays a critical role in tumor immune evasion and should be considered as an attractive target for therapeutic intervention.

Pembrolizumab (previously known as MK-3475 or SCH 900475) is a potent and highly selective humanized monoclonal antibody (mAb) of the IgG4/kappa isotype designed to directly block the interaction between PD-1 and its ligands, PD-L1 and PD-L2.

An open-label Phase I trial (Protocol 001) was conducted to evaluate the safety and clinical activity of single agent MK-3475. The dose escalation portion of this trial evaluated three dose levels, 1 mg/kg, 3 mg/kg, and 10 mg/kg, administered every 2 weeks (q2wk) in subjects

with advanced solid tumors. All three dose levels were well tolerated and no dose-limiting toxicities were observed. This first in human study of MK-3475 showed evidence of target engagement and objective evidence of tumor size reduction at all dose levels (1 mg/kg, 3 mg/kg and 10 mg/kg q2wk). No MTD was identified.

PK data analysis of MK-3475 administered q2wk and q3wk showed slow systemic clearance, limited volume of distribution, and a long half-life (see also pembrolizumab IB). Pharmacodynamic data (IL-2 release assay) suggested that peripheral target engagement is durable (>21 days).

A population pharmacokinetic analysis has been performed using serum concentration time data from 476 patients. Within the resulting population PK model, clearance and volume parameters of MK-3475 were found to be dependent on body weight. The relationship between clearance and body weight, with an allometric exponent of 0.59, is within the range observed for other antibodies and would support both body weight normalized dosing or a fixed dose across all body weights. MK-3475 has been found to have a wide therapeutic range based on the melanoma indication. The differences in exposure for a 200 mg fixed dose regimen relative to a 2 mg/kg q3wk body weight based regimen are anticipated to remain well within the established exposure margins of 0.5 – 5.0 for MK-3475 in the melanoma indication. The exposure margins are based on the notion of similar efficacy and safety in melanoma at 10 mg/kg q3wk vs. the proposed dose regimen of 2 mg/kg q3wk (i.e. 5-fold higher dose and exposure). The population PK evaluation revealed that there was no significant impact of tumor burden on exposure. In addition, exposure was similar between the NSCLC and melanoma indications. Therefore, there are no anticipated changes in exposure between different indication settings.

Thus the choice of the 200 mg q3wk as an appropriate dose for the switch to fixed dosing is based on simulations performed using the population PK model of pembrolizumab showing that the fixed dose of 200 mg every 3 weeks will provide exposures that 1) are optimally consistent with those obtained with the 2 mg/kg dose every 3 weeks, 2) will maintain individual patient exposures in the exposure range established in melanoma as associated with maximal efficacy response and 3) will maintain individual patients exposure in the exposure range established in melanoma that are well tolerated and safe.

A fixed dose regimen simplifies the dosing regimen to be more convenient for physicians and to reduce potential for dosing errors. A fixed dosing scheme will also reduce complexity in the logistical chain at treatment facilities and reduce wastage.

Background combination dabrafenib+trametinib in BRAF mutant melanoma

In vitro and in vivo preclinical data indicated increased anti-tumor activity of a combination of the BRAF-inhibitor dabrafenib with the MEK inhibitor trametinib. The combination of dabrafenib and trametinib has demonstrated enhanced anti-proliferative activity against a panel of BRAF-mutant cell lines in vitro, suggesting a synergistic effect of dabrafenib and trametinib in addressing primary resistance to each single agent. In addition, the combination was also effective in inhibiting the growth of dabrafenib resistant BRAF-mutant melanoma

cell clones indicating the potential ability of the combination therapy to overcome acquired resistance. This cell-line data generated by GlaxoSmithKline (GSK) were comparable to recently published in vitro results of other experimental BRAF- and MEK-inhibitor combinations [30, 31].

Moreover, the combination of dabrafenib and trametinib demonstrated improved activity in mouse xenograft models of BRAF-mutant melanoma compared to either single agent. Furthermore, in skin toxicity studies performed in rats, the addition of trametinib to dabrafenib prevented the development of proliferative skin lesions observed following treatment with dabrafenib alone. These results suggests that the addition of a MEK inhibitor to a BRAF inhibitor may suppress the proliferative signals in normal skin cells which can lead to the development of hyperproliferative skin lesions including keratoacanthomas and cutaneous squamous-cell carcinomas frequently observed in clinical trials involving BRAF-inhibitors. [21, 32, 33]. Similar results have been published with another combination of BRAF and MEK inhibitors [8]. While reducing the skin related adverse event rates, the combination of BRAF and MEK inhibition has been shown to improve progression free survival rates in about 50% in late stage BRAFV600 mutation positive melanoma patients [8, 33, 34].

Background translational research

Tumors such as melanoma are thought to be immunogenic tumors and therefore are responsive to T cell checkpoint blockade. Indeed, many melanoma-associated antigens have been identified, and T cell responses against them described regularly in melanoma patients [35-38]. Recently it has been shown, that response upon PD-1 blockade is associated with increased CD8+ tumor T cell infiltration [39], and that within the PD-1+ CD8+ intratumoral T cell pool the melanoma neo-antigen-specific T cells can be found, that correlates with the peripheral PD-1+CD8+ T cells [40, 41]. This technique allows an indirect analysis of changes of melanoma-specific T cell responses in peripheral blood mononuclear cells (PBMCs), normally restricted to HLA-A2 positive patients [42]. In addition, an interferon (IFN) signature has been associated with favorable outcome upon pembrolizumab treatment [43]. By comparing intra-patient changes in T cell infiltrations in the tumor, percentage of PD-1+CD8+ peripheral T cells in peripheral blood, and RNA-signatures upon therapy, we expect that the ability to pick up differences is much higher as compared to a setting in which clinically only interpatient comparisons are feasible. This analysis will be performed initially on pre-, week 6, week 12, and week 18 peripheral blood mononuclear cell (PBMC) samples of patients in all groups.

In addition EDTA blood will be preserved for later thrombocyte analyses. Tumor-educated thrombocytes (TeT) have been recently shown to be representative for different malignancies, but their significance as predictive marker for response is not yet known [44].

Rationale for exploring E-nose as possible biomarker

The Spironose is an e-Nose produced by the AMC (Amsterdam) and Common Invent (BV, Delft, The Netherlands), which can be used in combination with routine lung function

testing.[45] It is currently used for the development of a non-invasive diagnostic tool to discriminate COPD, asthma and lung cancer by analyzing the exhaled gasses. Earlier research showed the Spironose is able to discriminate between COPD and lung Cancer (De Vries et al. Poster ERS 2015). In this study, with 55 COPD patients and 43 lung cancer patients, the positive predictive value was 94%. Furthermore, 13 patients with lung cancer were classified as COPD, however 10 of these 13 lung cancer patients did have COPD as well. Preliminary data of a pilot study in patients with lung cancer treated with nivolumab suggests that early discrimination of responders and non-responders (at 6 weeks after initiation of treatment) might be possible [data unpublished].

In this study we aim to explore the value of the e-Nose as a biomarker for prediction of response to immunotherapy in melanoma.

Rationale for intermittent addition of dabrafenib+trametinib to pembrolizumab

The contrasting response patterns (BRAF inhibitors: high RR, but short PFS; PD-1 or CTLA-4 blockade: lower RR, but considerable long-term benefit within responding patient group) have early led to the idea of combining immune- and targeted therapy. Indeed, selective BRAF inhibition has been shown to increase T cell tumor infiltration in melanoma patients transiently [46, 47] and to improve T cell stimulation when co-cultured with BRAF inhibitor pretreated melanoma cell lines [48].

However, initial attempts of combining continuous application of vemurafenib or dabrafenib + trametinib with ipilimumab failed due to toxicity ([9], and Puzanov et al., abstract #2511, ASCO 2014). Recently, it has been shown, that continuous application of dabrafenib, trametinib, and anti-PD-L1 (MEDI4736, durvalumab) is well safe and induces increased tumor T cell infiltration on day 15 (Ribas et al., abstract #3003, ASCO 2015). However, it has also been shown that longer application of BRAF inhibitors results in disappearance of this increased T cell infiltration ([47], and our own so far unpublished observation). Thus we postulate that intermittent/short term BRAF+MEK inhibition will be the best adjuvans for pembrolizumab treatment. Within the different cohorts the patients will be stratified according to baseline LDH levels, as this has been shown to be one of the strongest biomarkers for response upon PD-1 blockade (Larkin et al. ECC 2015, and Weide, Blank et al., manuscripts submitted). For pembrolizumab it has been determined that the response rate was for patients with normal LDH about 44%, and for patients with increased LDH about 22%, while PD-L1 negative patients responded in about 10%, and PD-L1 positive (>1% PD-L1 staining) in about 40% [49]. Unfortunately PD-L1 staining has not been standardized so far.

Continuous combination of BRAF+MEK inhibition and PD-L1 blockade has been shown to be safe and to induce increased T cell infiltrates at week 2 (Ribas et al, ASCO 2015). However, as discussed above, there is growing evidence that beyond 4-6 weeks of targeted therapies these increased T cell tumor infiltration disappears.

Thus, we postulate that combined short-term BRAF+MEK inhibition would better synergize (by antigen retrieval and increase of intratumoral immune infiltrates) with pembrolizumab. In line with this clinical observations, we found in our mouse model of human melanoma [10, 11], short-term BRAF+MEK inhibition (7 days) to induce the highest CD8+ T cell tumor

infiltration and the most favorable intratumoral CD8+/Treg ratios, when comparing different targeted therapy combinations, inclusive single BRAF inhibition (Blank et al., manuscript in preparation). In addition, high CD8+ T cell melanoma infiltration has been correlated with response upon pembrolizumab in two independent cohorts [39].

We have observed in one patient treated with a higher dose than the standard 2mg/kg pembrolizumab within the MK-3475-006 study (10mg/kg), who switched short after 4 courses to dabrafenib+trametinib strong immune activation, as manifested by fever up to 41°C, immune related meningitis symptoms (recovering within an hour after steroid substitution). Interestingly, this patient, who suffered from fast progression of omental cake, ascites, and pleural effusion after the 4 courses of pembrolizumab, was in near CR after 7 weeks of treatment with dabrafenib + trametinib for several months, despite no treatment at all after onset of the irAEs. This one case indicates that there could be synergy between dabrafenib, trametinib and pembrolizumab, but also indicates that safety still needs to be monitored as primary outcome, despite the available data of dabrafenib, trametinib, and PD-L1 blockade (MEDI4736, durvalumab), being declare to be safe (Ribas et al., abstract #3003, ASCO 2015).

In summary, preclinical data, and clinical observations, indicate that BRAF+MEK inhibition can increase CD8+ T cell tumor infiltration, a prerequisite for response upon pembrolizumab. Furthermore, these changes occur very fast, arguing against the need for continuous combination of targeted therapy and immunotherapy that has in theory the potential to be toxic, and even counterproductive (decreasing the tumor T cell infiltration).

In this trial we will therefore address the question, whether intermittent/short-term dabrafenib+trametinib application during pembrolizumab treatment increases the tumor immune infiltration further, whether it increases response rate and progression-free survival, and whether it induces new unknown adverse events.

Risk benefit discussion

Pembrolizumab monotherapy and the combination of dabrafenib and trametinib have both been tested as save and effective treatment options for melanoma patients [2, 12], and have become approved standard therapies for late stage melanoma. Also a combination of anti-PD-L1 and dabrafenib+trametinib has been tested to be safe (Ribas et al., abstract #3003, ASCO 2015).

Nevertheless, short term combination of dabrafenib and trametinib with PD-1/PD-L1 antibodies could increase again the rate of immune related adverse events, as we assume that they will not revert the initial induction of tumor immune infiltrates, as seen in continuous BRAF+MEK inhibition beyond 6 weeks.

Thus an additional burden for the patients of participating in this trial could be an increased chance of developing more frequent or more severe immune related adverse events.

Early after BRAF+MEK inhibition a strong increase of CD8+ T cells infiltration has been observed, also when combined with PD-L1 blockade (Ribas et al., abstract #3003, ASCO 2015). Considering, the fact that a higher melanoma CD8+ T cell infiltration has been associated with higher response rates upon pembrolizumab [39], we postulate that such triplet therapy can benefit the patients concerning response rate and progression free survival.

Another additional burden for the patients participating in this trial in all cohorts are the additional tumor biopsies that will be taken from easy accessible lesions (lymph node or subcutaneous lesions, which presence is an inclusion criteria), and amount of blood drawing (the frequency is identical to standard pembrolizumab monotherapy, but the volumes taken are much higher).

2. OBJECTIVES

Primary objectives:

To explore the safety, feasibility, and the immune-activating capacity of different schemes of continuous/intermittent dabrafenib+trametinib during treatment with pembrolizumab as compared to pembrolizumab monotherapy

Primary endpoints:

- Safety and feasibility as measured by SUSARs and adherence to the timelines of the study protocol (week 0 till week 18).
- Alterations in percentage of tumor infiltrating CD8+ T cells and in percentage of PD-1+CD8+ T cells in peripheral blood samples in the time interval pre-treatment to week 18 intrapatient, and interpatient, pembrolizumab only (cohort 1) versus pembrolizumab plus intermittent dabrafenib/ trametinib (cohorts 2-4).

Secondary objectives:

- To determine rates of response at week 6, 12, week 18, and best overall response.
- To determine progression-free survival starting from randomization.
- To determine long-term toxicities of intermittent dabrafenib + trametinib during treatment with pembrolizumab as compared to pembrolizumab monotherapy

Secondary endpoints:

- Rates of response at week 6, week 12, week 18, and best overall response, according to RECIST 1.1 criteria
- Progression-free survival (PFS) starting from randomization to progression using RECIST 1.1 criteria.
- Rate and type of late adverse events (beyond week 18, for up to 2 years)

Explorative objectives:

- To explore the value of the e-Nose as an early biomarker for prediction of response to treatment.
- To describe time to progression beginning from week 12 (cohorts 2-4).
- Describe changes of immune parameters within the tumor.

Exploratory endpoints:

- As the short addition of dabrafenib+trametinib will induce for short time tumor regressions we will analyze cohorts 2-4 with a second baseline, namely the end of the targeted therapy at week 12, for progression free survival using to RECIST 1.1

- In addition to the primary readout (broadening of the melanoma-specific T cell response in peripheral blood), we will analyze the effect of the different therapy schemes on tumor immune cell infiltration (IHC for CD3, CD4, CD8, CD68, FoxP3, PD-L1, PD-L2, PD-1, CD11b, HLA) and perform RNA sequencing to determine changes in immune/IFN signatures [43].

- The accuracy with which an eNose can discriminate between responders and non-responders at week 6 and 12, as compared to baseline.

3. STUDY DESIGN

This is a Phase 2 trial consisting of patients that harbor a BRAFV600mut positive melanoma, of which 24 patients will receive the combination of dabrafenib + trametinib + pembrolizumab (cohorts 2-4), and 8 patients (cohort 1) receiving pembrolizumab standard monotherapy. Latter will be included for explorative material collection (Figure 1). To ensure comparability all patients need to meet the same study inclusion criteria, and will be randomized to one of the cohorts at week 6, stratified according to baseline LDH level (normal, > ULN, or > 2xULN).

Figure 1

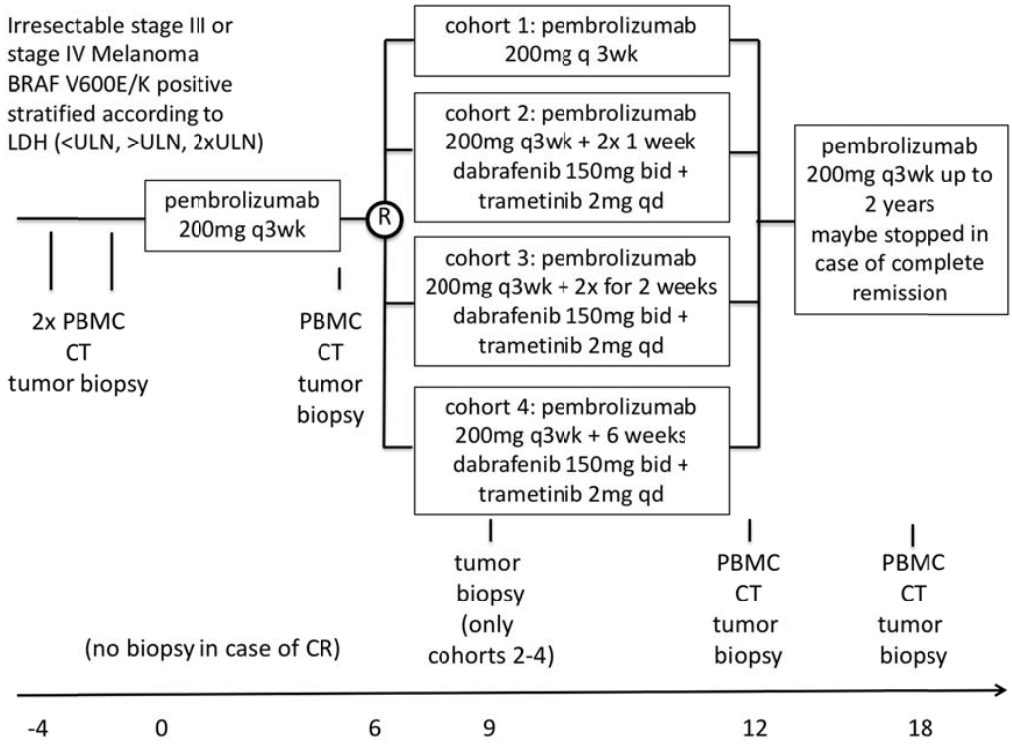


Figure 1: Schematic overview of IMPemBra study.

Considering the fact that a maximum continuous dosing of dabrafenib + trametinib and the anti-PD-L1 monoclonal antibody MEDI4736 was well tolerated, all cohorts will be randomized in parallel. Nevertheless a combination cohort (cohorts 2-4) shall be set on hold if in the unlikely case 2 of the first 5 patients perceive DLTs attributed to the triple combination therapy within the first 18 weeks.

4. STUDY POPULATION

4.1 Study Population

Irresectable stage III or stage IV BRAFV600E/K mutation positive melanoma patients, naïve for CTLA-4/PD-1/PD-L1 immunotherapy, more than 18 years old.

4.2 Inclusion criteria

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

- Adults at least 18 years of age
- World Health Organization (WHO) Performance Status 0-2
- Irresectable stage III or stage IV melanoma
- Histologically/cytologically confirmed BRAF V600E or K melanoma
- Measurable disease according to RECIST 1.1
- At least one easy accessible lesion (s.c., lymph node) that can be repeatedly biopsied
- Patient willing to undergo triple tumor biopsies during screening, at week 6, week 9 (cohorts 2-4 only), week 12, at week 18, and in case of disease progression.
- No prior immunotherapy targeting CTLA-4, PD-1, or PD-L1 (except for adjuvant therapy)
- No prior BRAF and/or MEK targeting therapy
- No immunosuppressive medications
- Screening laboratory values must meet the following criteria:

  WBC ≥ 2.0×10^9/L, Neutrophils ≥ 1.0×10^9/L, Platelets ≥ 100×10^9/L, Hemoglobin ≥ 5.0 mmol/L

  Creatinine ≤ 2x ULN

  AST, ALT ≤ 2.5 x ULN (≤5 x ULN for patients with liver metastases)

  Bilirubin ≤2 X ULN
- Female subject of childbearing potential should have a negative urine or serum pregnancy within 72 hours prior to receiving the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.
- Women of child bearing potential must agree to use a reliable form of contraceptive during the study treatment period and for at least 120 days following the last dose of study drug
- Men must agree to the use of male contraception during the study Treatment Period and for at least 180 days after the last dose of study drug.

4.3 Exclusion criteria

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

- Is currently participating in or has participated in a study of an investigational agent or using an investigational device within 4 weeks of the first dose of treatment.
- Known brain metastases, that have not been treated before inclusion and have not been confirmed to be stable for at least 6 weeks
- Leptomeningeal metastases
- Prior CTLA-4/PD-1/PD-L1 targeting immunotherapy (except if given as adjuvant therapy)
- Has an active autoimmune disease requiring systemic treatment within the past 3 months or a documented history of clinically severe autoimmune disease, or a syndrome that requires systemic steroids or immunosuppressive agents. Subjects with vitiligo or resolved childhood asthma/atopy would be an exception to this rule. Subjects that require intermittent use of bronchodilators or local steroid injections would not be excluded from the study. Subjects with hypothyroidism stable on hormone replacement or Sjorgen's syndrome will not be excluded from the study.
- Has had a prior monoclonal antibody within 4 weeks prior to study day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier.
- Has had prior chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to a previously administered agent.
    - Note: Subjects with ≤ Grade 2 neuropathy are an exception to this criterion and may qualify for the study.
    - Note: If subject received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.
- Live vaccines within 30 days prior to the first dose of study therapy and while participating in the study.
- Has evidence of interstitial lung disease or active, non-infectious pneumonitis.
- Known history of Human Immunodeficiency Virus;
- Active infection requiring therapy, positive tests for Hepatitis B surface antigen or Hepatitis C;
- Has active tuberculosis
- Has a known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin, squamous cell carcinoma of the skin, or in situ cervical cancer that has undergone potentially curative therapy. Patients that have had another malignancy, but are free of tumor for more than 2 years are allowed for inclusion.

- Underlying medical conditions that, in the Investigator's opinion, will make the administration of study drug hazardous or obscure the interpretation of toxicity determination or adverse events;
- Concurrent medical condition requiring the use of immunosuppressive medications, or
- Immunosuppressive doses of systemic or absorbable topical corticosteroids
- Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial.

4.4 Sample size calculation

One of the primary endpoints of the study is in particular the safety and feasibility of intermittent/short-term continuous dabrafenib+trametinib during immunotherapy with pembrolizumab. However, this needs to be contrasted to a therapy where pembrolizumab is given as standard monotherapy. The optimal way to gain experience with these approaches is by randomizing in a phase 2 design. We therefore propose to randomize 32 patients to either receiving pembrolizumab monotherapy, or different schemes of dabrafenib+trametinib.

A cohort will be defined as not safe and feasible, if 2 out of the first 5 patients (point estimate 0.4 (95%CI 0.05-0.85)) in each of the triple combination cohorts (cohorts 2-4) will experience immune-related adverse events unknown for pembrolizumab monotherapy (see IB pembrolizumab), leading non-adherence to the study scheme (except for irAEs being already present at week 6).

The investigators realize that numbers of immune-related adverse events smaller than respectively 2 out of 5 still bare a substantial chance of error of taking the wrong conclusion about safety, but they do not really expect adverse events other than one described before for pembrolizumab.

The number of 8 patients in each arm is chosen with the focus on producing relevant numbers of T cell responses that can be analyzed (immune-activating capacity). In patients with metastatic melanoma, melanoma specific T cell responses are observed in the majority of patients (>80%) treated by ipilimumab [50].

Considering that a) the rate of response for pembrolizumab is about 3-fold higher than the one upon ipilimumab (33.7% versus 11.9%) [1], b) it has been shown, that response upon PD-1 blockade is associated with increased CD8+ tumor T cell infiltration [39], and c) that within the PD-1+ CD8+ intratumoral T cell pool the melanoma neo-antigen-specific T cells can be found, that correlates with the peripheral PD-1+CD8+ T cells [40, 41], we expect to define possible changes in every patient. Furthermore, this indirect technique allows analysis of changes of melanoma-specific T cell responses in PBMC in all patients because it is not restricted to HLA-A2 positive patients [42].

Another aim of this study is to explore and tentatively detect the activity of different schedules of the combination of dabrafenib+trametinib added to pembrolizumab after induction with pembrolizumab with respect to PFS. After 6 weeks of pembrolizumab treatment, patients will be randomized between standard continuation with pembrolizumab treatment and 3 different treatment schemes of the combination (cohorts 2-4): adding 1 week, 2 weeks or short-term continuous dabrafenib+trametinib.

Each of the experimental cohorts will consist of 8 patients and will be evaluated primarily on their own. In each cohort, eight patients will be accrued over a period of 1 year and followed for another 2 years. This number of patients will allow the detection of a hazard ratio of 0.5 using a one-sided α of 0.15 and a power of 80%. This assumes an improvement in median PFS from 3 to 6 months. The control group (cohort 1) will provide a reference value for the median PFS. In case that the PFS reference value is clearly different from the assumption, the three cohorts together can still be contrasted to the median PFS of the standard cohort.

The comparison between standard and experimental treatment in 32 patients in 4 cohorts (1 standard compared to the 3 experimental groups together) would provide about 70-80% power (alpha 0.15) to detect a hazard of 0.5 with small deviations in the assumed median PFS.

4.5 Evaluations by confirmed RECIST 1.1 criteria

All measurements in this study will be performed according to RECIST 1.1 guidelines [51]. Time to progression will be measured starting at week 6 (baseline) and confirmed in case of progression by another measurement 4-6 weeks later.

At baseline, tumor lesions/lymph nodes will be categorised measurable or non-measurable as follows:

Measurable

- Tumour lesions: Must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of:
- 10 mm by CT scan (CT scan slice thickness no greater than 5 mm).
- 10 mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measurable).
- 20 mm by chest X-ray.

Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be P15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed (see Schwartz et al. in this Special Issue15). See also notes below on 'Baseline documentation of target and non-target lesions' for information on lymph node measurement.

Non-measurable

- All other lesions, including small lesions (longest diameter <10 mm or pathological lymph nodes with P10 to <15 mm short axis) as well as truly non-measurable lesions. Lesions considered truly non-measurable include: leptomeningeal dis- ease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

Response Criteria

- Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.
- Partial Response (PR): At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.
- Progressive Disease (PD): At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).

- Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study

5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

Patients will be treated with 200mg pembrolizumab i.v., every 3 weeks, for up to 2 years. Patients achieving a complete response may interrupt treatment after 6 months of treatment and re-induce treatment if disease recurrence is observed.

Patients in cohorts 2 and 3 will receive additionally two courses intermittent of dabrafenib + trametinib p.o. during the week 7-9 and week 10-12 (between the pembrolizumab infusions) and in cohort 4 one continuous course of 6 weeks of dabrafenib + trametinib in week 7-12:

- cohort 1: pembrolizumab 200mg i.v., q 3weeks
- cohort 2: pembrolizumab + dabrafenib 150mg bid + trametinib 2mg qd, 2 x for 1 week
- cohort 3: pembrolizumab + dabrafenib 150mg bid + trametinib 2mg qd, 2 x for 2 weeks
- cohort 4: pembrolizumab + dabrafenib 150mg bid + trametinib 2mg qd, 1x for 6 weeks

All cohorts continue pembrolizumab for up to two years in case of achieving confirmed clinical benefit (CR, PR, or SD, according to RECIST 1.1, see 4.5) at week 18.

Drug holiday is allowed in both study parts in case of CR and treatment with pembrolizumab for at least 6 months. Re-induction in case of disease recurrence is permitted.

Patients with confirmed progression at week 18 go off study, but will be followed for late toxicity every 3 months for additional 6 months.

A total of 32 patients (8 patients standard monotherapy pembrolizumab and 24 patients in the combination cohorts) will be included into this study.

5.2 Use of co-intervention

Prohibited medication:

1) Concurrent chemotherapy, hormonal therapy, immunotherapy regimens, or radiation therapy, standard or investigational.
2) Use of growth factors including, but not limited to, granulocyte colony stimulating factor (G-CSF), granulocyte macrophage colony stimulating factor (GM-CSF), or erythropoietin stimulating agents are not permitted, unless deemed necessary by investigator and discussed with medical monitor.
3) Use of systemic corticosteroids at > 10 mg daily prednisone equivalent, unless required for the treatment of infusion reactions, other adverse events, or for palliation as determined by the investigator.
4) Use of RANKL therapy or bisphosphate therapy
5) Steroids must not be given as prophylactic anti-emetic therapy.
6) Use of herbal remedies is not permitted

Permitted medication:

The use of prescription and over-the-counter medications (except medications from categories outlined above) is permitted at the discretion of the Investigator and must be recorded on CRF.

Subjects are permitted the use of topical, ocular, intranasal, intra-articular, and inhalational corticosteroids (with minimal systemic absorption). Immunosuppressive doses (e.g., prednisone > 1mg/kg/day or equivalent) and/or physiologic replacement doses of systemic corticosteroids (e.g., prednisone ≤ 10 mg/day) are permitted in the context of treating adverse events. A brief course of corticosteroids for prophylaxis (e.g., contrast dye allergy) or for treatment of non-autoimmune conditions (e.g., delayed-type hypersensitivity reaction caused by a contact allergen) is permitted.

Prophylactic anti-emetics, with the exception of steroids, may be administered at the discretion of the treating physician before any doses of study drug.

Use of the seasonal killed influenza vaccine during therapy is permitted without restriction. However, influenza vaccines containing live attenuated virus (Flumist ®) or other clinically indicated vaccinations for infectious diseases (killed or attenuated, e.g., Pneumovax ®, varicella, MMR, etc) may be permitted, but must be discussed with the Principal investigator and may require a study drug washout period prior to and after administration of the vaccine.

Contraception:

A Woman of Childbearing Potential (WOCBP) is defined as any female who has experienced menarche and who has not undergone surgical sterilization (hysterectomy or bilateral oophorectomy) and is not postmenopausal. Menopause is defined as 12 months of amenorrhea in a woman over age 45 years in the absence of other biological or physiological causes. In addition, females under the age of 55 must have a serum follicle stimulating hormone, (FSH) level > 40mIU/mL to confirm menopause.

Females treated with hormone replacement therapy, (HRT) are likely to have artificially suppressed FSH levels and may require a washout period in order to obtain a physiologic FSH level. The duration of the washout period is a function of the type of HRT used. The duration of the washout period below are suggested guidelines and the investigators should use their judgment in checking serum FSH levels. If the serum FSH level is > 40 mIU/ml at any time during the washout period, the woman can be considered postmenopausal:

- 1 week minimum for vaginal hormonal products, (rings, creams, gels)
- 4 week minimum for transdermal products
- 8 week minimum for oral products

Other parenteral products may require as long as 6 months.

5.3 **Escape medication (if applicable)** n.a.

6. INVESTIGATIONAL PRODUCT

6.1 Name and description of investigational product(s)

Drug	Dose	Dose Frequency	Route of Administration	Treatment Period	Use
pembrolizumab	200mg	q 3wk	i.v. infusion	up to 2 years	cohort 1-4
dabrafenib	cohort 2,3,4: 150mg bid	bid	p.o.	cohort 2: 2x for 1 week cohort 3: 2x for 2 weeks cohort 4: 6 weeks	cohort 2-4
trametinib	cohort 2,3,4: 2mg	Qd	p.o.	cohort 2: 2x for 1 week cohort 3: 2x for 2 weeks cohort 4: 6 weeks	cohort 2-4

Trial treatment should begin on the day of registration or as close as possible to the date on which treatment is allocated/assigned.

6.2 Preclinical Summary of Pembrolizumab

See page 12 and pembrolizumab IB

6.3 Summary of findings from clinical studies

Pembrolizumab Monotherapy

See page 12 and pembrolizumab IB

Combination of dabrafenib and trametinib

See page 17 and dabrafenib and trametinib IB

6.4 Summary of known and potential risks and benefits

Pembrolizumab is a T cell checkpoint blocker, and thus immune related adverse events in every organ could occur. So far grade 3 and 4 toxicities have been rare. Further details can be obtained from the pembrolizumab IB.

The combination of dabrafenib and trametinib is also well tolerated. Interestingly skin toxicity was seen less often as compare to dabrafenib monotherapy [34]. More often observed than with dabrafenib monotherapy were chills that often can be controlled by paracetamol. Further details can be obtained from the dabrafenib IB and trametinib IB.

The combination of continuous application of dabrafenib+trametinib and pembrolizumab has been meanwhile been tested in a phase 1 trial (15 patients, Keynote-022 study, ASCO 2016). 66.7% of the patients observed grad 3 and 4 toxicities and in 3/14 evaluable patients DLT was observed. The recommended phase 2 regimen became pembrolizumab 2 mg/kg q3wks + dabrafenib 150 mg bid + trametinib 2 mg qd, a scheme similar to the one of arm 4 in this trial. As of the data cutoff date, there were 9 objective responses, 5 of which were confirmed (all partial responses); 2 additional patients had stable disease.

6.5 Description and justification of route of administration and dosage

Pembrolizumab is a monoclonal antibody and need to be i.v. infused. Dosing in this trial is a flat dose application identical to the recommended phase 2 dosing. See also page 12.

Dabrafenib and trametinib are applied p.o. Maximum dosing in this trial is identical to recommended phase 2 dosing that has been also used in the phase 3 trials [7, 21].

6.6 Dosages, dosage modifications and method of administration

Pembrolizumab

Pembrolizumab will be administered as a 30 minute IV infusion (treatment cycle intervals may be increased due to toxicity as described in IB). Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps from site to site, a window of -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes: -5 min/+10 min). In case of intolerability of 30 min infusion, the extension of the infusion time to up to 90 min is allowed.

Details regarding the mixing and concentrations of the dose (preparation) and administration will be found in the current pembrolizumab procedures manual and the IB.

Dabrafenib plus trametinib

Dabrafenib and trametinib will be applied at standard dose of dabrafenib 150mg bid, and trametinib 2mg qd, as tested to be safe and effective in several randomized phase 2 and 3 trials [21, 33, 34]. Dose modifications are not expected to be implemented due to the short application of dabrafenib+trametinib in this trial (2x 1-2 weeks, and 1x 6 weeks). If needed dabrafenib and trametinib will be stopped (e.g. in case of severe immune related adverse

events in combination with pembrolizumab). Please refer to the dabrafenib and trametinib IB for further details.

6.7 Dose delay criteria

Pembrolizumab will be withheld for drug-related Grade 4 hematologic toxicities, non-hematological toxicity ≥ Grade 3 including laboratory abnormalities, and severe or life-threatening AEs as per Table 3 below.

Table 3: Dose modification guidelines for drug-related adverse events.

Toxicity	Grade	Hold Treatment (Y/N)	Timing for restarting treatment	Dose/Schedule for restarting treatment	Discontinue Subject (after consultation with Sponsor)
Hematological Toxicity	1, 2	No	N/A	N/A	N/A
	3* *Excluding Grade 3 neutropenia, anemia, and thrombocytopenia	Yes	Toxicity resolves to Grade 0-1 or baseline	May increase the dosing interval by 1 week	Toxicity does not resolve within 6 weeks of last infusion
	4	Yes	Toxicity resolves to Grade 0-1 or baseline	May increase the dosing interval by 1 week	*Permanent discontinuation should be considered for any severe or life-threatening event*
Non-hematological toxicity Note: Exception to be treated similar to grade 1 toxicity • Grade 2 alopecia • Grade 2 fatigue For additional information regarding Adverse Events with a potential Immune-Etiology reference appendix	1	No	N/A	N/A	N/A
	2	Consider withholding for persistent symptoms	Toxicity resolves to Grade 0-1 or baseline	*Clinical AE resolves within 4 weeks*: Same dose and schedule (reference appendix for recommendations regarding pneumonitis) *Clinical AE does not resolve within 4 weeks:* May increase the dosing interval by 1 week for each occurrence	Toxicity does not resolve within 6weeks of last infusion

Toxicity	Grade	Hold Treatment (Y/N)	Timing for restarting treatment	Dose/Schedule for restarting treatment	Discontinue Subject (after consultation with Sponsor)
	3, 4	Yes	Toxicity resolves to Grade 0-1 or baseline	May increase the dosing interval by 1 week for each occurrence	Toxicity does not resolve within 12 weeks of last infusion *Permanent discontinuation should be considered for any severe or life-threatening event*

In case toxicity does not resolve to Grade 0-1 within 6 weeks (within 3 weeks during first 12 weeks of the trial) after last infusion, trial treatment should be discontinued after consultation with the Sponsor. With investigator and Sponsor agreement, subjects with a laboratory adverse event still at Grade 2 after 12 weeks may continue treatment in the trial only if asymptomatic and controlled.

Subjects who experience a recurrence of the same severe or life-threatening event at the same grade or greater with re-challenge of pembrolizumab should be discontinued from trial treatment.

6.8 Criteria to resume treatment

Subjects may resume treatment with study drug when the drug-related AE(s) resolve to Grade ≤1 or baseline value, with the following exceptions:

- Subjects may resume treatment in the presence of Grade 2 fatigue
- Subjects who have not experienced a Grade 3 drug-related skin AE may resume treatment in the presence of Grade 2 skin toxicity
- Subjects with baseline Grade 1 AST/ALT or total bilirubin who require dose delays for reasons other than a 2-grade shift in AST/ALT or total bilirubin may resume treatment in the presence of Grade 2 AST/ALT OR total bilirubin
- Subjects with combined Grade 2 AST/ALT AND total bilirubin values meeting discontinuation parameters (Section 6.9) should have treatment permanently discontinued
- Drug-related pulmonary toxicity, diarrhea, or colitis, must have resolved to baseline before treatment is resumed

- Drug-related endocrinopathies adequately controlled with only physiologic hormone replacement may resume treatment

If the criteria to resume treatment are met, the subject should restart treatment at the next scheduled timepoint per protocol/standard scheme. However, if the treatment is delayed past the next scheduled timepoint per protocol, the next scheduled timepoint will be delayed until dosing resumes. If treatment is delayed > 6 weeks, the subject must be permanently discontinued from study therapy, except as specified in discontinuation section.

6.9 Discontinuation criteria

Treatment should be permanently discontinued for the following:

- Any Grade 2 drug-related uveitis or eye pain or blurred vision that does not respond to topical therapy and does not improve to Grade 1 severity within the re-treatment period OR requires systemic treatment
- Any Grade 3 non-skin, drug-related adverse event lasting > 7 days, with the following exceptions for drug-related laboratory abnormalities, uveitis, pneumonitis, bronchospasm, diarrhea, colitis, neurologic adverse event, hypersensitivity reactions, and infusion reactions
    - Grade 3 drug-related uveitis, pneumonitis, bronchospasm, diarrhea, colitis, neurologic adverse event, hypersensitivity reaction, or infusion reaction of any duration requires discontinuation
    - Grade 3 drug-related laboratory abnormalities do not require treatment discontinuation except those noted below
        - Grade 3 drug-related thrombocytopenia > 7 days or associated with bleeding requires discontinuation
        - Any drug-related liver function test (LFT) abnormality that meets the following criteria require discontinuation:
            - AST or ALT > 8 x ULN
            - Total bilirubin > 5 x ULN
            - Concurrent AST or ALT > 3 x ULN and total bilirubin > 2 x ULN
- Any Grade 4 drug-related adverse event or laboratory abnormality, except for the following events which do not require discontinuation:
    - Isolated Grade 4 amylase or lipase abnormalities that are not associated with symptoms or clinical manifestations of pancreatitis and decrease to < Grade 4 within 1 week of onset.
    - Isolated Grade 4 electrolyte imbalances/abnormalities that are not associated with clinical sequelae and are corrected with supplementation/appropriate management within 72 hours of their onset
- Any dosing interruption lasting > 6 weeks with the following exceptions:

- Dosing interruptions to allow for prolonged steroid tapers to manage drug-related adverse events are allowed. Prior to re-initiating treatment in a subject with a dosing interruption lasting > 6 weeks, the Investigator must be consulted. Tumor assessments should continue as per protocol even if dosing is interrupted
- Dosing interruptions > 6 weeks that occur for non-drug-related reasons may be allowed if approved by the Investigator. Prior to re-initiating treatment in a subject with a dosing interruption lasting > 6 weeks, the Investigator must be consulted. Tumor assessments should continue as per protocol even if dosing is interrupted

- Any adverse event, laboratory abnormality, or intercurrent illness which, in the judgment of the Investigator, presents a substantial clinical risk to the subject with continued pembrolizumab dosing
- Confirmed progression according to RECIST1.1 at week 18. In the case that the treating physician suspects a late response, treatment beyond progression for another 6 weeks is permitted.
- Confirmed progression after initial response or disease stabilization in comparison with the nadir (not baseline).

6.10 Treatment of pembrolizumab related infusion reactions

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for < =24 hrs	**Stop Infusion and monitor symptoms.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of MK-3475 with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of antipyretic).

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
	If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g. from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDS Acetaminophen Narcotics Oxygen Pressors Corticosteroids Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **Subject is permanently discontinued from further trial treatment administration.**	No subsequent dosing
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration. For Further information, please refer to the Common Terminology Criteria for Adverse Events v4.0 (CTCAE) at http://ctep.cancer.gov.		

6.11 Preparation and labeling of Investigational Medicinal Product

Packaging and labeling of pembrolizumab will be done by MSD.

6.12 Drug accountability

Will be done by the pharmacy of the NKI.

7. METHODS

7.1 Study parameters/endpoints

7.1.1 Main study parameter/endpoint

Primary readout will be safety and feasibility, and the exploration of the alteration inpercentage of CD8+ intratumoral T cells and of PD-1+CD8+ T cells in peripheral blood samples in the time interval pre- to during therapy (week 6, 12, and 18).

Failure of feasibility of one of the cohorts is defined as non-adherence to the study scheme in the cohorts 2-4 between week 6 and 18 in more than 2 patients within the first 5 patients per cohort (except for irAE being already present at week 6).

7.1.2 Secondary study parameters/endpoints

Rate and type of late adverse events Response rates and progression free survival of the combination of pembrolizumab with intermittent or short-term dabrafenib+trametinib PFS, as determined by to RECIST 1.1 criteria.

7.1.3 Other study parameters (if applicable)

Alterations of the intratumoral immune cell infiltrates and RNA signatures.

Progression free survival using week 12 as baseline (only cohorts 2-4)

7.2 Registration, blinding and treatment allocation

Patients fulfilling the eligibility criteria will be enrolled in the study. After signing informed consent the patients will first be registered for screening. When they fulfill all eligibility criteria the patient will be registered in the study and can start the pembrolizumab treatment. After 6 weeks (2 infusions) the patients will be randomized to 1 of the 4 cohorts.

7.3 Study procedures

An overview of the study procedures can also be found in Table 2.

7.3.1 Screening phase

Following signing of the informed consent form for screening and enrolment into the study, the remainder of screening procedures and tests will be completed.

- Complete physical examination including WHO PS, height, weight, temperature, pulse, blood pressure
- Tumor biopsies for pathologic confirmation of stage III or IV melanoma and genetic analysis of tumor. Patients may enter the study with a pathologic diagnosis of melanoma from any institution, confirmed by revision of the NKI-AVL Department of Pathology. If the

diagnosis is not confirmed, the patient will be excluded from the study and replaced by another patient. New biopsies must be obtained then only for baseline material asservation.

- BRAF-status, if not yet determined.
- Hb, ANC, and platelet count, incl. differentiation, Hct, LDH, phosphorus, sodium, potassium, magnesium, chloride, calcium, creatinine, albumin, total protein, SGOT (AST), SGPT (ALT), bilirubin (ind + dir), GGT, alkaline phosphatase, TSH, T4, ACTH, cortisol, LH, FSH, testosterone/oestradiol, S100, CRP, ESR.
- Baseline diagnostic CT of the chest, abdomen and pelvis to evaluate the status of disease, within 4 weeks before registration.
- MRI brain, if clinically indicated
- ECG, if clinically indicated
- HIV antibody titer, HbsAg determination, and Anti-HCV.
- beta-HCG pregnancy test on all women of child-bearing potential
- PBMC's (100 mL heparinized blood), EDTA blood (10ml), and serum (10 mL) will be taken twice before start of treatment.
- E-Nose analysis

7.3.2 During treatment

- The patients will be treated with 200mg flat dose pembrolizumab, every 3 weeks.
- At week 6 the patients will be randomized (and stratified according to baseline LDH: normal, >1xULN, or >2xULN) to continue with pembrolizumab monotherapy (cohort 1) or to receive in addition 2x for 1 or 2 weeks dabrafenib+trametinib in between week 6-9 and week 9-12 (cohorts 2 and 3). or to receive in addition for 6 weeks dabrafenib+trametinib in week 7-12 (cohort 4).
- Targeted physical examination and measurement of weight, temperature, pulse, and blood pressure will be performed in week 3, 6, 9, 12, and 18, and every 3 months
- Hb, ANC, and platelet count, incl. differentiation, Hct, LDH, phosphorus, sodium, potassium, magnesium, chloride, calcium, creatinine, albumin, total protein, SGOT (AST), SGPT (ALT), bilirubin (ind + dir), GGT, alkaline phosphatase, TSH, T4, ACTH, cortisol, LH, FSH, testosterone/oestradiol, S100, CRP, ESR before every pembrolizumab infusion
- beta-HCG pregnancy test on all women of child-bearing potential, every second infusion.
- CT of the chest, abdomen and pelvis week 6, 12 and week 18, and subsequently every 3 months
- From all patients PBMC's (100 mL heparinized blood), EDTA blood and serum (each 10 mL) will be taken during treatment at 6, 12 and 18 of the study, and subsequently every 3 months on treatment.

- Tumor biopsies (3x 14G) at week 6, week 9 (cohort 2-4 only; this additional biopsy is added due to the likely chance that at week 6 of targeted therapy no lesion for biopsy is anymore present), week 12, and week 18 (exclusive patients achieving complete remission of all easy accessible lesion)
- E-Nose analysis week 6 and week 12

7.3.3 Post treatment evaluation

Patients with clinical benefit will be evaluated every 3 months by targeted physical examination and lab testing (same as on treatment), until year 3. Subsequent structured follow-up will be according to the current melanoma guidelines (year 4 and 5 every 6 months, year 6 - 10 once a year).

In case of tumor relapse or progression tumor biopsies (3x14G), PBMC, EDTA blood, and serum collection will be done.

7.4 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.

7.4.1 Specific criteria for withdrawal

If after having signed informed consent but before initiation of the treatment, patients do not fulfill the eligibility criteria anymore, patients will be withdrawn from the study.

7.5 Replacement of individual subjects after withdrawal

Patients that are withdrawn prior to initiation of the treatment and patients that do not fulfill the eligibility criteria will be replaced.

7.6 Follow-up of subjects withdrawn from treatment

Follow-up of patients withdrawn from the study will continue at least until 100 days after the treatment or until the treatment related toxicity has resolved to grade 2 or less (CTCAE 4.0). Thereafter, patients will receive standard follow-up for the disease.

7.7 Premature termination of the study

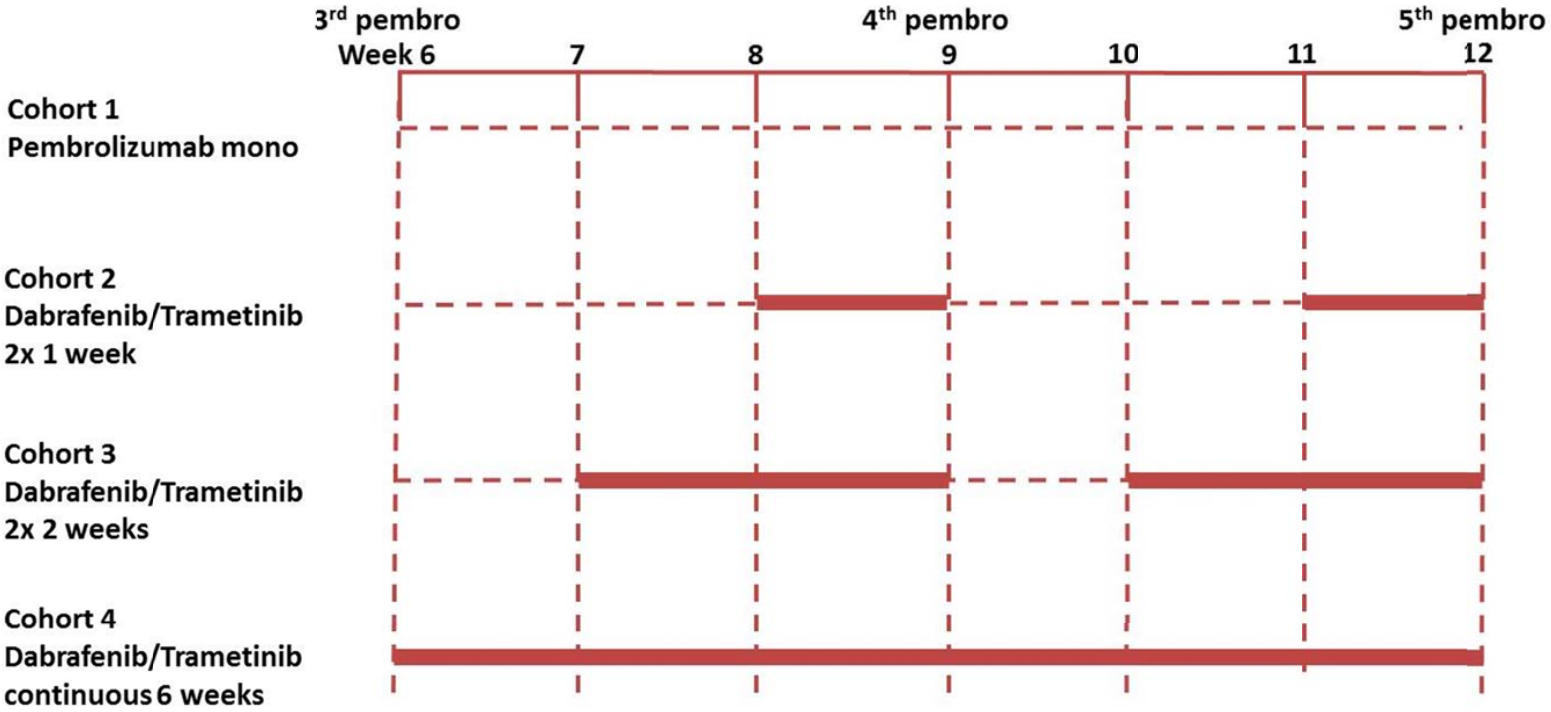
In case of unexpected toxicity or feasibility the sponsor will discuss premature termination of the study with the ethical committee and with MSD.

Table 2. Schedule of assessments[1]	**Screening Week -4 - BL**	**Treatment BL Week 0**	**Week 3**	**Week 6**	**Week 9**	**Week 12**	**Week 15**	**Week 18**	**On treatment[10]**	**Follow-up[2]**
Informed consent	x									
PA confirmation stage III/IV melanoma	x									
BRAF status	x									
Medical history	x									
Physical examination[3]	x	x	x	x	x	x	x	x	x	x
Hematology[4]	x	x	x	x	x	x	x	x	x	x
Chemistry[5]	x	x	x	x	x	x	x	x	x	x
beta-HCG pregnancy test	x	x		x		x		x	x[10]	
Serology[6]	x									
MRI brain*	x									
CT chest, abdomen, pelvis[#]	x			x		x		x	x[10]	x
ECG*	x									
Adverse events[7]		x	x	x	x	x	x	x	x	x
PBMCs, EDTA, serum[8]	x	x		x		x		x	x[10]	x
Tumor biopsy[9]	x			x	x[9]	x		x		x[9]
e-Nose	x			x		x				

* if clinically indicated, [#] at baseline within 4 weeks
1 For treatment schedule see section 6.1
2 Follow-up of off-study patients until 100 days post last treatment, follow-up of responders every 3 months until 3 years, then according to standard guidelines (www.oncoline.nl)
3 Targeted physical examinations, incl. WHO PS, weight, temperature, pulse, blood pressure
4 Hb, ANC, and platelet count, incl. differentiation, Hct
5 LDH, phosphorus, sodium, potassium, magnesium, chloride, calcium, creatinin, albumin, total protein, SGOT (AST), SGPT (ALT), bilirubin (ind + dir), GGT, alkaline phosphatase, TSH, T4, ACTH, cortisol, LH, FSH, S100, CRP, ESR.

6 HIV, HbsAG, HCV
7 AEs will be graded according to CTCAE 4.0
8 100 ml heparinized blood + 10 ml EDTA blood + 10 ml serum
9 Three biopsies taken with a 14G needle, during screening, week 6, week 9 (cohorts 2-4 only), week 12, and week 18 (only lymph node or s.c. metastases). During follow-up only at timepoint of relapse/disease progression.
10 Physical examination, AE documentation, Hematology and Chemistry before every pembrolizumab infusion, pregnancy test only every 6 weeks, CT scans and PBMC only every 3 months

Treatment schedule



8. Safety reporting

8.1.1 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.

8.2 AEs, SAEs, ECIs, and SUSARs

8.2.1 Adverse events (AEs)

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

8.2.2 Serious adverse events (SAEs)

A Serious Adverse Event (SAE) is any untoward medical occurrence that at any dose:

- results in death
- is life-threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- requires inpatient hospitalization or causes prolongation of existing hospitalization (see note below)
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- is an important medical event (defined as a medical event(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the subject or may require intervention [eg, medical, surgical] to prevent one of the other serious outcomes listed in the definition above.) Examples of such events include, but are not limited to, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization). Hospitalization for infusions, e.g. erythrocytes, steroids, infliximab, are not considered SAEs.
- Potential drug induced liver injury, renal failure, or pneumonitis are also considered an important medical event.
- Suspected transmission of an infectious agent (eg, pathogenic or nonpathogenic) via the study drug is an SAE.

- Although pregnancy, overdose, and cancer are not always serious by regulatory definition, these events must be handled as SAEs.
- Progression of the cancer under study is not considered an adverse event unless it results in hospitalization or death.

8.2.3 Events of Clinical Interest (ECIs)

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be reported to Merck Global Safety.

For the time period beginning when the consent form is signed until treatment allocation/randomization, any ECI, or follow up to an ECI, that occurs to any subject must be reported within 24 hours to Merck Global Safety if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation/randomization through 30 days following cessation of treatment, any ECI, or follow up to an ECI, whether or not related to Merck's product, must be reported within 24 hours to Merck according to the SAE reporting guidelines.

Events of clinical interest for this trial include:

1. an overdose of Merck's product, that is not associated with clinical symptoms or abnormal laboratory results.

2. an elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.

8.2.4 Reporting of SAEs and ECIs

All Serious Adverse Events (SAE) occurring from start of pembrolizumab treatment until 100 days after the last protocol treatment/administration should be documented and reported (the study specific SAE form should be used) immediately to the NKI-AVL Safety Desk by fax: 0031 (0)20-5122679 between 09.00 and 17.00 hours Monday to Friday. If a fax is not possible due to technical problems, the Data Centre should be contacted by telephone: 0031 (0)20-5122668) between 09.00 and 17.00 hours Monday to Friday.

The Safety Officer will notify the study coordinator immediately of any serious adverse event (as defined above) experienced by a patient. The study coordinator will evaluate the SAE and will decide whether the event reported could be related to the protocol treatment and whether it is, both, unexpected and serious (SUSAR, see 8.2.4.).

The NKI-AVL Safety Desk will report the SAE's by fax to the subsidizing party Merck Global Safety facsimile number: +1-215-993-1220.

SAEs will be followed up by the safety department of the AVL. Updates on the SAE will be sent to MSD similarly within 2 days.

In addition, serious adverse events will be reported by the NKI-AVL Safety Desk to the METC and the CCMO. All SAE's will be reported once yearly, as described in the section 8.3. SAE's will not be reported through the web portal ToetsingOnline to the METC.

Serious adverse events occurring more than 100 days after the last study medication will NOT be reported unless the investigator feels that the study drug or a protocol procedure may have caused the event.

Any adverse event leading to hospitalization or prolongation of hospitalization will be considered as 'serious', UNLESS at least one of the following exceptions are met:

• the admission is pre-planned (e.g. elective or scheduled surgery, documented in the patient's file);

• hospitalization for technical (e.g. study drug administration) , practical or social reasons, in the absence of an adverse event.

Common toxicities observed for progressive disease and events secondary to progressive disease are generally excluded from reporting. However, in cases where the specificity or severity of an event is not consistent with the risk information, the event should be reported.

All SAE reports will be filed in the Investigator Study File.

In case the SAE is unexpected a SUSAR will be reported (see section 8.2.4).

Non-serious Events of Clinical Interest (ECI) will also be forwarded to Merck Global Safety and will be handled in the same manner as SAEs.

8.2.5 Reporting of Pregnancy and Lactation to the Sponsor and to Merck

Although pregnancy and lactation are not considered adverse events, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them), including the pregnancy of a male subject's female partner that occurs during the trial or within 100 days of completing the trial completing the trial, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier. All subjects and female partners of male subjects who become pregnant must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious

events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

Such events must be reported within 24 hours to the Sponsor and within 2 working days to Merck Global Safety (attn: worldwide product safety; fax +1-215 993-1220).

8.2.6 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 8.2.2);

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 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.

8.3 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.

8.4 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 will to be reported till 100 days after discontinuation of the study drug.

8.5 [Data Safety Monitoring Board (DSMB) / Safety Committee]

No DSMB will be set-up.

9. STATISTICAL ANALYSIS

9.1 Statistical plan

9.1.1 Efficacy analysis

see 4.4

9.1.2 Safety analysis

The All-Patients-as-Treated population will be employed for safety analyses. Treatment related adverse experiences are pre-specified as events of interest.

9.2 Power and Sample size

see 4.4

9.3 Interim analysis

An interim analysis is not planned.

9.4. Definitions Endpoints

see 2.

10. ETHICAL CONSIDERATIONS

10.1 Regulation statement

This study will be conducted according to the principles of the Declaration of Helsinki, (Declaration of Helsinki, 59th WMA General Assembly, Seoul, October 2008) and in accordance with the Medical Research Involving Human Subjects Act (WMO). The protocol has been written, and the study will be conducted according to the ICH Harmonized Tripartite Guideline for Good Clinical Practice (ref:http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6_R1/ Step4/E6_R1__Guideline.pdf).

The protocol must be approved by the CCMO and competent authority (CA).

10.2 Recruitment and consent

Informed consent (IC)

All patients will be informed of the aims of the study, the possible adverse events, the procedures and possible hazards to which he/she will be exposed, and the mechanism of treatment allocation. They will be informed as to the strict confidentiality of their patient data, but that their medical records may be reviewed for trial purposes by authorized individuals other than their treating physician.

It will be emphasized that the participation is voluntary and that the patient is allowed to refuse further participation in the protocol whenever he/she wants. This will not prejudice the patient's subsequent care. Documented informed consent will be obtained for all patients included in the study before they are registered in the study. This will be done in accordance with the national and local regulatory requirements.

The IC procedure will conform to the ICH guidelines on Good Clinical Practice. This implies that "the written IC form will be signed and personally dated by the patient or by the patient's legally acceptable representative".

Recruitment

It is the responsibility of the investigator to give each patient, prior to inclusion in the trial, full and adequate verbal and written information regarding her rights, the objective and procedures of the trial and the possible risks involved. Confidentiality-related information will be provided. The written patient information must be given to each patient. Patients will be given sufficient time for consideration. An independent physician will be available in accordance with the requirements of the national law. It is the responsibility of the investigator to obtain signed informed consent from every patient prior to the start of any study related procedure. The written patient information is part of the documentation reviewed by the MEC mentioned. The patient information letter and informed consent form are attached as a separate document.

10.3 Benefits and risks assessment, group relatedness

See page 17

10.4 Compensation for injury

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

The NKI-AVL (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.

1. € 450.000,-- (i.e. four hundred and fifty thousand Euro) for death or injury for each subject who participates in the Research;

2. € 3.500.000,-- (i.e. three million five hundred thousand Euro) for death or injury for all subjects who participate in the Research;

3. € 5.000.000,-- (i.e. five million Euro) for the total damage incurred by the organisation for all damage disclosed by scientific research for the Sponsor as ‘verrichter’ in the meaning of said Act in each year of insurance coverage.

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

10.5 Incentives (if applicable)

The patients will not receive any incentive for study participation, including no compensation for travelling.

11. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

11.1 Subject identification

Following enrolment a patient sequential identification number or code will be allocated to the patients. This number will be used for identification of the patients and should be reported on all case record forms (CRFs). Data and patient material will be handled confidentially and if possible anonymously. When it is necessary to trace data or material to an individual subject, we will use a subject identification number list to link the data to the subject. The code is based on the sequence of enrolment combined with month-year of the patients' birth date. The key to the code will be safeguarded by the Principle Investigator. The handling of personal data complies with the Dutch Personal Data Protection Act (in Dutch: De Wet Bescherming Persoonsgegevens, WBP).

11.2 Registration of the patients

Patient registration will only be accepted from authorized investigators or through their authorized data manager or authorized staff member. A patient can be registered only after verification of eligibility. During the registration procedure eligibility criteria and items of patients' informed consent are checked. Registration will be done by the Trial Office of the AVL after receiving the original signed IC and after verification of the eligibility criteria.

11.3 Storage of patient material

Patient material will be stored in the research laboratory to be used for additional research upon new research developments or insights.

11.4 Data management

All data that are relevant for the study will be collected on eCRFs developed by the datacenter of the AVL. The completed eCRFs must be reviewed and signed by the principal investigator or sub-investigator.

11.5 Monitoring and Quality Assurance

Source data verification of the CRFs and check of the Investigator Study File documents will be performed by the clinical research monitor of the NKI-AVL, according to the procedures described in the Monitor Plan.

11.6 Amendments

All amendments will be notified to the METC that gave a favorable opinion.

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 investigator.

11.7 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.

11.8 End of study report

The investigator 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 investigator 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 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.

11.9 Public disclosure and publication policy

Prior to initiation, the study will be submitted to the Dutch National Trial register, which is a recognized and accepted by the World Health Organization and International Committee of Medical Journal Editors (ICMJE) and to the NCI's PDQ® Cancer Clinical Trials Registry. All the results will officially be published.

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