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Name of Sponsor/Company: Universitätsklinikum Heidelberg		**Sponsor-Code of Study:** CheCUP, CA209-8WY	*(For National Authority Use only)*
Name of (Finished) Product: OPDIVO® 10 mg/ml YERVOY® 5mg/ml		**Name of Active Ingredient:** Nivolumab Ipilimumab	
EudraCT-No.: 2018-004562-33	**CA Vorlage-No.:** 3824	**IEC Antrags-No.:** AFmu-530/2019	

☒ **End of Trial Report** ☐ **Annual Safety Report**

SYNOPSIS

Title of Study:
A phase II, open-label, non-randomized, multi-center study evaluating the efficacy and safety of nivolumab plus ipilimumab in patients with cancer of unknown primary site who are relapsed after or refractory to platinum-based chemotherapy (CheCUP)

Date of Approval / Vote: initial protocol version V1.7
PEI: 04.12.2019 **Ethics Committee:** 27.11.2019

Amendments:
1) Protocol amendment v1.8 and corresponding ICFs
Date of Approval / Vote:
PEI: 07.08.2020 **Ethics Committee:** 13.08.2020
Rationale: Changes to inclusion and exclusion criteria to enhance subject enrolment e.g. extension of screening period, acceptance of pre-existing TMB analysis (not older than 6 months) or pre-existing tumor FFPE tissue blocks, if a re-biopsy failed for inclusion, inclusions of subjects with limited brain metastases and COVID-19 triggered changes e.g. allowance of local blood sample analysis and possible remote patient visits, if no IMP is administered.

2) New Nivolumab and Ipilimumab Investigator brochures
Date of Approval / Vote:
PEI: 31.08.2020 **Ethics Committee:** 13.08.2020
Rationale: Release of new IB editions: Nivolumab IB version 19, Ipilimumab IB version 23

3) Protocol amendment v1.9 and corresponding ICFs
Date of Approval / Vote:
PEI: 15.12.2020 **Ethics Committee:** 22.12.2020
Rationale: clarifications and changes to inclusion and exclusion criteria to enhance subject enrolment e.g. allowance of up to two year old pre-existing tumor FFPE tissue blocks, if re-biopsy was unsuccessful.

4) New Nivolumab and Ipilimumab Investigator brochures
Date of Approval / Vote:
PEI: 28.09.2021 **Ethics Committee:** 28.09.2021
Rationale: Release of new IB editions: Nivolumab IB version 20, Ipilimumab IB version 24

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EudraCT-No.:	CA Vorlage-No.:	IEC Antrags-No.:
2018-004562-33	3824	AFmu-530/2019

Investigators:
Coordinating Investigator (LKP): Prof. Dr. med. Alwin Krämer
Deputy Investigator: PD Dr. med. Tilmann Bochtler

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Trial site Essen
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EudraCT-No.:	CA Vorlage-No.:	IEC Antrags-No.:
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Trial site: Tübingen
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Trial site: Gütersloh
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Trial site: Berlin
was positively voted by the leading and participating ethic committees, however site was never initiated and enrolled patients.

Publication (reference):
Not applicable

Study period: (date of first enrolment) (date of last completed)	12.12.2019 – 15.03.2022	Phase of development:	II

Objectives:
Primary:
To compare the efficacy of nivolumab plus ipilimumab in subjects with high (≥ 12 mutations/MB) vs. Intermediate/low (< 12 mutations/MB) TMB (tumor mutational burden) poor-prognosis CUP (non-specific subset) who are relapsed or refractory to platinum-based first-line chemotherapy. The Progression-free survival (PFS), defined as the time from treatment start to the first occurrence of disease progression, was assessed according to Response Evaluation Criteria in Solid Tumors, Version 1.1 (RECIST v1.1), or death from any cause, whichever occurred first.

Secondary:
To evaluate the efficacy of nivolumab plus ipilimumab in subjects with poor-prognosis CUP (non-specific subset) who are relapsed or refractory to platinum-based first-line chemotherapy by analysis of the overall survival (OS), the overall response rate (ORR) and the duration of clinical benefit (DCB).

Safety:
To evaluate the safety of nivolumab and ipilimumab treatment by analysing the incidence, nature and severity of adverse events (AEs) and the incidence and reasons for any dose reductions, interruptions or premature discontinuation of study treatment.

Methodology:
This trial is a prospective, non-randomized, multicentred, open-label phase II clinical trial in patients with cancer of unknown primary site who relapsed after or were refractory to platinum-based chemotherapy.
The tumor mutational burden (TMB) was used as biomarker for stratification into TMB-high versus TMB-intermerdiate/low groups. Subjects showing high TMB (cut-off 12 mutations/Mb) were considered biomarker-positive and expected to have a favourable prognosis for progression-free survival when treated with immune checkpoint inhibitors. 15% of subjects diagnosed with CUP syndrome are expected to be biomarker-positive. Subjects were planned to be enrolled in a high vs. TMB intermediate/low 1:1 ratio. From the 31 patients recruited into the trial 5 patients were TMB-high (16%).

Number of Volunteers (planned and analysed):
Planned: 194, enrolled/analysed: 31
The subject recruitment was terminated prematurely due to slow enrolment. Enrolled subjects were treated and followed-up according to the protocol. All enrolled subjects were evaluable for efficacy and safety.

Diagnosis and main criteria for inclusion:
CUP-syndrome, relapsed/refractory to platinum-based chemotherapy, ICD10: C80.0

Key inclusion criteria:

- Signed Informed Consent Form
- Able and willing to comply with the study protocol
- Age ≥ 18 years at time of signing Informed Consent Form
- Histologically-confirmed disseminated or advanced unresectable CUP diagnosed according the criteria defined in the 2015 ESMO Clinical Practice Guidelines for CUP. Acceptable disease histology includes:
  - Adenocarcinoma of unknown primary site (ACUP)
  - Poorly differentiated adenocarcinoma of unknown primary site
  - Poorly differentiated carcinoma of unknown primary site
  - Squamous cell carcinoma of unknown primary site (SCUP)
- At least one lesion that is measurable according to RECIST v1.1 by CT/MRI
- Availability of a tumor FFPE block either fresh or archival if obtained ≤ 6 months at Screening that is sufficient for generation of a TruSight Oncology 500 (TSO500) panel at the central

reference pathology laboratory or pre-existing result of a TMB analysis from routinely performed panel sequencing using the TSO500 panel at the MPZ, Institute of Pathology, University Heidelberg, which must not be older than < 6 months at screening, respectively. In case one attempt to perform TMB analysis on a new specimen has failed due to insufficient tumor cell quantity or insufficient quality in the specimen, or a re-biopsy has failed or cannot be performed for clinical or technical reasons, resorting to a specimen not older ≤24 months is allowed as an exception.

- Availability of test reports confirming local CUP diagnosis. If test reports confirming local CUP diagnosis are not available, an FFPE block or a fresh biopsy sample must be submitted that is sufficient to allow for central confirmation of CUP diagnosis.
- Disease relapse or progression after at least three cycles of a platinum-based standard chemotherapy. There is no upper limit of prior treatments received.
- Subjects who have received prior surgery and/or radiotherapy and/or stereotactic brain metastasis radiosurgery are eligible. In case of prior radiotherapy, the measurable lesion(s) must not have been irradiated, radiotherapy has to be finished at least 7 days before start of study treatment and the patient must have recovered to grade 1 or less from any toxicity of radiotherapy.
- ECOG performance status of 0 - 2
- Life expectancy ≥ 12 weeks
- Eligible for immune checkpoint inhibitor
- Adequate hematologic and end-organ function as detailed in the protocol (see section 4.4)
- For women of childbearing potential and men capable of reproduction: agreement to remain abstinent (refrain from heterosexual intercourse) or use contraceptive methods with a failure rate of < 1% per year during the treatment period and for at least 5 months for women and 7 months for men, respectively after the last dose of study treatment.
- Recovery from significant toxicity from platinum-doublet therapy to Grade ≤ 1, except for alopecia and for neurosensory toxicity, which must be ≤ 2
- Recovery from active infections requiring intravenous antibiotics, with antibiotic therapy ceased for ≥ 7 days prior to planned start of therapy

<u>Key Exclusion Criteria:</u>

- Subjects with any of the specific non-CUP neoplasms identified in the ESMO CUP guidelines (Fizazi et al. 2015)
- Subjects belonging to any of the following subsets of CUP with favorable prognoses:
  – Poorly differentiated carcinoma with midline distribution
  – Women with papillary adenocarcinoma of the peritoneal cavity
  – Women with adenocarcinoma involving only the axillary lymph nodes
  – Squamous cell carcinoma restricted to cervical lymph nodes
  – Poorly and well differentiated neuroendocrine tumors
  – Men with blastic bone metastases and elevated PSA
  – Subjects with a single, small tumor potentially resectable and/or amenable to radiotherapy with curative intent
  – Colon cancer-type CUP
- Known presence of brain or spinal cord metastasis, as determined by CT or magnetic resonance imaging (MRI) evaluation during screening. As an exception, patients with brain

metastases are allowed to be included if all of the following five criteria are met:
(i) the total number of brain metastases is 3 or less,
(ii) brain metastases were / are asymptomatic,
(iii) brain metastases have been completely surgically resected or completely treated with stereotactic radiosurgery
(iv) there was / is no indication for whole-brain irradiation,
(v) a brain MRI or high-resolution CT-scan at screening shows no evidence of residual disease.
If 1 to 3 asymptomatic brain metastases are detected at screening and are treatable and treated with stereotactic radiosurgery within the screening period, no renewed MRI / CT imaging of the brain is required before inclusion.
Benign lesions such as meningiomas may be accepted, if demonstration is made that they will not affect the interpretation of the study results or render the patient at high risk from treatment complications.

- History or known presence of leptomeningeal disease
- Uncontrolled or symptomatic hypercalcemia (serum calcium $\geq$ 2.9mmol/L)
- Known clinically significant history of liver disease consistent with Child-Pugh Class B or C, including active viral or other hepatitis, current alcohol abuse, or cirrhosis
- Human immunodeficiency virus (HIV) infection
- Positive for hepatitis C virus (HCV) infection at screening
- Positive for hepatitis B surface antigen (HBsAg) at screening
- Active tuberculosis at Screening
- Significant cardiovascular disease (such as New York Heart Association Class II or greater cardiac disease, myocardial infarction, or cerebrovascular accident) within 3 months prior to initiation of study treatment, unstable arrhythmia (including active ventricular arrhythmia requiring medication), or unstable angina
- Major surgical procedure, other than for diagnosis, within 4 weeks prior to initiation of study treatment, or anticipation of need for a major surgical procedure during the study
- History of malignancy other than CUP within 5 years prior to screening, with the exception of malignancies with a negligible risk of metastasis or death (e.g., 5-year OS rate > 90%), such as adequately treated carcinoma in situ of the cervix, non-melanoma skin carcinoma, localized prostate cancer, ductal carcinoma in situ, or stage I uterine cancer
- Solid organ transplantation
- Prior allogeneic stem cell transplantation with follow-up < 1 year, need for systemic immunosuppression or active chronic graft-versus host disease (cGVHD)
- Any other disease, metabolic dysfunction, physical examination finding, or clinical laboratory finding that contraindicates the use of an investigational drug, may affect the interpretation of the results, or may render the patient at high risk from treatment complications
- Known allergy or hypersensitivity to any component of the immunotherapy, including history of severe allergic anaphylactic reactions to chimeric or humanized antibodies or fusion proteins and to Chinese hamster ovary cell products or other recombinant human or humanized antibodies for nivolumab and ipilimumab.
- Subjects with an active autoimmune disease, including, but not limited to, myasthenia gravis, myositis, autoimmune hepatitis, myocarditis, systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, antiphospholipid antibody syndrome, Wegener

granulomatosis, Sjögren syndrome, Guillain-Barré syndrome, or multiple sclerosis. Subjects with type I diabetes mellitus, hypothyroidism only requiring hormone replacement, skin disorders (such as vitiligo, psoriasis, or alopecia) not requiring systemic treatment, or conditions not expected to recur in the absence of an external trigger are permitted to enroll.

- Subjects with a condition requiring systemic treatment with either corticosteroids (> 10 mg daily prednisone equivalents), or other immuno-suppressive medications within 14 days of study treatment. Inhaled or topical steroids and adrenal replacement doses > 10 mg daily prednisone equivalents in the absence of active autoimmune disease are permitted.
- Subjects who received prior treatment with an anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CD137, or anti-CTLA-4 antibody, or any other antibody or drug specifically targeting T-cell co-stimulation or checkpoint pathways
- All toxicities attributed to prior anti-cancer therapy other than alopecia and fatigue must have resolved to Grade 1 (NCI CTCAE version 5) or baseline before administration of study drug. Subjects with toxicities attributed to prior anti-cancer therapy which are not expected to resolve and result in long lasting sequelae, such as neuropathy after platinum-based therapy, are permitted to enroll.
- Systemic treatment for cancer (any chemotherapy, biologics for cancer or investigational therapy) within 21 days of first administration of study treatment
- Radiotherapy or stereotactic brain metastasis radiosurgery has to be finished at least 7 days before inclusion into the study and the subject must have recovered to grade 1 or less from any toxicity of radiotherapy / stereotactic brain metastasis radiosurgery.
- Subjects must not have received a live / attenuated vaccine within 30 days of first treatment.
- Pregnancy or breastfeeding, or intention of becoming pregnant during study treatment or within 5 months after the last dose of study treatment or intention of fathering a child within 7 months after the last dose of study treatment.

Test product (IMP being tested), trade name, MA holder, MA number, dose and mode of administration, batch number(s):

IMP 1: Trade name	YERVOY®
Active substance INN	Ipilimumab
Active substance - sponsor code	BMS-734016
Active substance - EV substance code	SUB29397
ENR/ONGNR	2670878
MA holder	Bristol-Myers Squibb Pharma EEIG
MA number	EU/1/11/698/002
Dose and mode of administration	5 mg/ml ipilimumab concentrated solution for infusion, 1 mg/kg ipilimumab was administered as 30-minute infusion every 6 weeks
Batch numbers used:	AAV9534, ABW6020, ABE3010, AAX6418

IMP 2: Trade name	OPDIVO®
Active substance INN	Nivolumab
Active substance - sponsor code	BMS-936558
Active substance EV substance code	SUB122750
ENR/ONGNR	2672461
MA holder	Bristol-Myers Squibb Pharma EEIG
MA number	EU/1/15/1014/002
Dose and mode of administration	10 mg/ml concentrated solution for infusion, 240 mg nivolumab was administered as 30-minute infusion every 2 weeks
Batch numbers used:	AAV7017, ABN0538, AAZ6313, ABB6752

Reference therapy (IMP used a comparator), trade name, MA holder, MA number dose and mode of administration:
Not applicable

Duration of treatment:
Subjects were treated with nivolumab as a 30-minute infusion, 240 mg flat dose every 2 weeks and ipilimumab as a 30-minute infusion, 1 mg/kg every 6 weeks, starting on Day 1, until progression, unacceptable toxicity, investigator or subject decision to withdraw from therapy or death, whichever occurs first. In cases where subjects benefitted clinically a continued study treatment beyond progression was an allowed as an exception.
TMB-high patients received a median of nine treatment cycles (range 1-18) as compared to two cycles (range 1-16) in the TMB-low group. Three patients were still on treatment at the time of data cut-off (March 15, 2022).

When nivolumab and ipilimumab were administered on the same day, separate infusion bags and filters were used for each infusion. Nivolumab was administered first. The second infusion with ipilimumab was started not sooner than 30 minutes after completion of the nivolumab infusion.
The study treatment could be delayed or permanently discontinued due to side effects of nivolumab and ipilimumab administration. If side-effects appeared to be Ipilimumab-related, Ipilimumab could be permanently discontinued and the subject received Nivolumab only. However, Ipilimumab administration without Nivolumab was not allowed.

Criteria for evaluation: (efficacy, safety)
<u>Primary Endpoint:</u>
Primary endpoint is Progression-free Survival (PFS). PFS is defined as the time from start of therapy to the first observation of disease progression or death due to any cause. If a subject is lost to follow up, progression-free survival is censored at the time of last documented efficacy. Disease progression is assessed by the investigator according to Response Evaluation Criteria in Solid

Tumors, Version 1.1 (RECIST v1.1),

Secondary efficacy endpoints:
Overall survival (OS) is defined as the time from start of therapy to death due to any cause. If a subject is lost to follow up, overall survival time is censored at the time of last contact.
Overall response rate (ORR), defined as the proportion of subjects who exhibit a CR or PR to study treatment on two consecutive occasions ≥ 4 weeks apart.
Duration of clinical benefit (DCB), defined as the time from the first occurrence of a CR, PR or SD after treatment start until disease progression or death from any cause, whichever occurs first.
Responses will be determined by the investigator according to RECIST v1.1.

Safety and Tolerability Endpoints:
• Incidence, nature and severity of adverse events (AEs)
• Incidence and reasons for any dose reductions, interruptions, or premature discontinuation of any component of study treatment
• Clinically significant laboratory values and vital signs
AEs are analyzed according to MEDDRA coding.

Analysis Population:
All patients enrolled into the trial had valid TMB assessment and received trial medication, i.e., there is only one analysis population for efficacy and safety.

Interim Analysis:
Due to early termination of recruitment no formal interim analysis of efficacy was performed as initially planned.

Statistical methods:
For the primary analysis the null hypothesis of no difference in PFS between TMB groups was tested using a two-sided log-rank test at a significance level of 5%. Secondary analyses of the primary endpoint comprised a multivariable Cox regression model including relevant prognostic factors.
The secondary endpoint OS was analyzed similarly to PFS. Fisher's exact test was used to compare response rates. Incidence and severity of adverse events were analyzed for the safety population on a per patient basis. Due to the premature discontinuation of the trial, all analyses were exploratory.

Summary – Conclusions:

Efficacy Results:
In the CheCUP trial (EudraCT No. 2018-004562-33), CUP patients relapsed or who were refractory after platinum-based chemotherapy received nivolumab and ipilimumab following stratification in high vs. low TMB. High TMB levels (>12 mutations/Mb) were found in 16% of patients. Overall response rate was 16% (95% CI: 6-34%) in the total vs. 60% (95% CI: 15-95%) in the TMB-high cohort. High TMB was associated with a clear trend for better PFS (18.3 vs. 2.4 months, p=0.056).

Safety Results:
Thirty-three serious adverse events (SAEs) were reported in total for this trial.
Thereof, eighteen events were classified as serious adverse reactions (SARs), i.e. either the reporting investigator or the sponsor considered them (at least possibly) related to either IMP. Those

eighteen SARs included twenty-one diagnoses, i.e. in some cases more the one diagnosis was documented. In general, the SARs reflected the known pattern of side effects of treatment with checkpoint inhibitors such as bone marrow depression and immune-related adverse reactions (irAR). Nevertheless, five events were classified as 'unexpected' reactions, i.e. they fulfilled all criteria of suspected unexpected serious adverse events (SUSARs) and, as such, underwent expedited reporting to the authorities. Altogether six diagnoses were contained in those five SUSARs, i.e. in one case more than one diagnosis was reported. Beside Neutropenia and Cerebrovascular accident (both given at preferred term (PT) level), some common symptoms of irAR or bone marrow depression could be found amongst the observed SUSARs (Ileus, Sepsis, General physical health deterioration, and Infectious pleural effusion; all events given at PT level).

Three patients had to permanently discontinue their trial treatment due to therapy-related toxicities. All three events were known irAR of checkpoint inhibitors (immune-related colitis, gamma GT elevation, and hepatitis).

Twenty-four patients died on study. Thereof, seven in the treatment phase and seventeen in the follow-up period. By far most common cause of death was disease progression (20 cases). The remaining four fatalities were due to suicide, sepsis (2 patients), and pulmonary artery embolism. One fatality, i.e. a fatal sepsis resulting from neutropenia, was considered possibly related to the trial treatment.

Taken together, no new safety signals were generated in the current trial as all observed SARs belonged to the known safety patterns of the IMPs. From the safety perspective the trial treatment appears acceptable in the investigated population.

Conclusion:

TMB-high CUP patients relapsed or refractory after platinum-based chemotherapy benefit from combined ICI treatment with nivolumab and ipilimumab.

Date of report: 03.03.2023

Reference:

Fizazi K, *et al.* Cancers of unknown primary site: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* **26 Suppl 5**, v133-138 (2015).