Synopsis

Sponsor:
Alcedis GmbH
Winchesterstraße 3
35394 Gießen
Phone: +49 (0) 641-94436-0
Fax: +49 (0) 641-94436-70
Email: info@alcedis.de

Investigational medicinal product: denosumab, nivolumab, pembrolizumab

Drug substance:
- denosumab, RANKL inhibiting antibody
- nivolumab, PD-1 inhibiting antibody (alone or in combination with ipilimumab, a CTLA-4 inhibiting antibody)
- pembrolizumab, PD-1 inhibiting antibody

Registration: EudraCT-Number 2016-001925-15

Study title: Evaluation of immunological effects of the RANKL-inhibitor denosumab when administered concurrently with PD1-blocking antibodies (nivolumab, pembrolizumab) in patients with metastatic malignant melanoma with bone involvement.

National Coordinating Investigator:
Prof. Dr. Ralf Gutzmer
Universitätsklinik für Dermatologie, Venerologie, Allergologie und Phlebologie
Mühlenkreiskliniken - Johannes Wesling Klinikum Minden
Hans-Nolte-Str. 1
32429 Minden
Phone: +49 (0) 571-790-4501
Fax: +49 (0) 571-790-29-4500
Email: Ralf.Gutzmer@Muehlenkreiskliniken.de

Study sites: 6 sites in Germany:
1. Prof. Dr. med. Imke Grimmelmann, Medizinische Hochschule Hannover, Klinik für Dermatologie, Allergologie und Venerologie, Carl-Neuberg-Str. 1, 30625 Hannover
2. Prof. Dr. med. Dirk Schadendorf, Universitätsklinikum Essen, Klinik und Poliklinik für Dermatologie, Venerologie und Allergologie, Hufelandstr. 55, 45147 Essen
3. Prof. Dr. med. Jessica Hassel, Universitätsklinikum Heidelberg, Dermatologie/NCT, Im Neuenheimer Feld 460, 69120 Heidelberg
4. Prof. Dr. med. Bastian Schilling, Universitätsklinikum Würzburg, Klinik und Poliklinik für Dermatologie, Venerologie und Allergologie, Josef-Schneider-Str. 2, 97080 Würzburg
5. Dr. med. Carsten Weishaupt, Universitätsklinikum Münster, Klinik für Hautkrankheiten - Allgemeine Dermatologie und Venerologie, Von-Esmarch-Straße 58, 48149 Münster
6. Prof. Dr. Ralf Gutzmer, Universitätsklinik für Dermatologie, Venerologie, Allergologie und Phlebologie, Mühlenkreiskliniken - Johannes Wesling Klinikum Minden, Hans-Nolte-Str. 1, 32429 Minden

Publication:
Not applicable.

First patient in: 16.04.2019 **Last patient out:** 30.08.2021	**Phase:** IV

Study objective(s): The primary objective of this trial was to evaluate immunological and biological effects of the combination of PD-1 and RANKL inhibition on circulating

peripheral blood mononuclear cells (PBMC, esp. activated T-cells), chemokines, and cytokines (e.g., interferon gamma).

Primary endpoints:

1) Dynamic changes in the numbers of central memory, effector memory, and/or effector T-cells in the circulating blood (based on expression of CD45RA, CD45RO, CCR7, CD62L, and TCF-1).
2) Dynamic changes in the concentration of cytokines and chemokines present in circulating blood.

Secondary endpoints:

- Overall response rate (ORR) at 12 weeks and 24 weeks as determined by RECIST 1.1[1] criteria.
- Response rate of bone metastases at 12 weeks and 24 weeks as determined by RECIST 1.1[1, 2] and bone scans.
- Occurrence of adverse events as assessed by NCI CTCAE version 5.0.

Trial design: open-label, prospective, multicenter, translational (biomarker-driven) phase IV study

Methods: Before therapy and after 4, 12, and 24 weeks on therapy peripheral blood samples were planned to be taken and analyzed for immune cell subsets as well as cytokine levels. If clinically feasible, also tissue samples of melanoma metastases were planned to be taken. The planned duration of study participation was 24 weeks after start of treatment followed by a 30-day safety follow-up.

Number of patients (planned and analyzed): 20 patients were planned, and 17 patients were included.

Diagnosis and key inclusion criteria: Patients diagnosed with inoperable metastatic stage IV melanoma with bone metastases and a planned therapy with immune checkpoint inhibitors (ICI) (without previous CTLA-4 or PD-1 inhibiting therapy).

Inclusion criteria:

1. Provision of tumor tissue from metastatic site of disease for biomarker analyses.
2. Inoperable metastatic stage IV melanoma.
3. Planned therapy with PD-1 blocking antibody (nivolumab or nivolumab + ipilimumab or pembrolizumab) and denosumab as standard of care (SoC).
4. Measurable disease according to RECIST1.1 and at least one documented bone metastasis
5. Age 18 years or above.
6. Written, informed consent.
7. Willingness and ability of subjects to comply with scheduled visits, treatment schedule, laboratory testing, and other requirements of the study.

[1] Results of tumor stagings were documented by the study sites followed by a central review of the documented data by the National Coordinating Investigator in collaboration with the investigator responsible for translational research leading to a final assessment of tumor response. Analysis of all endpoints based on tumor response is based on the results of this final assessment.

[2] As the response to treatment of bone metastases is typically rather slow, a differentiation in 12 and 24 weeks was not done. Instead, the analysis of endpoints regarding tumor response of bone metastases was performed considering all data available during each patient's individual observation period. Furthermore, differentiation between response and stable disease regarding bone metastases was difficult, as imaging techniques such as PET-CT were not consistently applied. Therefore, also patients with an apparent stable disease were counted as having response regarding bone metastases.

8. Minimum life expectancy of 6 months.
9. ECOG performance status of 0-2.
10. Screening laboratory values had to be obtained within 14 days prior to registration and had to meet the following criteria:
    - WBC ≥2000/µl
    - Neutrophils ≥1000/µl
    - Platelets ≥100.000/µl
    - Hemoglobin ≥9.0 g/dl
    - Serum creatinine ≤2.0 x ULN
      or creatinine clearance (CrCl) ≥ 35ml/min (using the Cockcroft-Gault formula)
    - AST/ALT ≤3 x ULN, in case of liver metastases ≤5 x ULN
    - Total Bilirubin ≤2.0 x ULN (except subjects with Gilbert Syndrome, who may have had total bilirubin up to 5 x ULN)
    - Serum calcium or albumin-adjusted serum calcium within normal limits
11. Prior radiotherapy had to be completed prior to study drug administration.
12. Negative pregnancy test for female subjects within the week before treatment start and effective contraception for both male and female subjects if the risk of conception existed.

<u>Exclusion Criteria</u>

Patients were excluded from the study for any of the following reasons:

1. Prior therapy with CTLA-4 inhibitor or PD-1 inhibitor or denosumab for distant metastatic disease. Treatments in the adjuvant setting were allowed in case treatment was discontinued at least 4 weeks before inclusion in this study.
2. No other concurrent medical treatments for metastatic disease such as targeted therapies or chemotherapies were allowed. Treatments in the adjuvant setting were allowed in case treatment was discontinued at least 4 weeks before inclusion in this study.
3. Active central nervous system (CNS) metastases requiring local therapy or steroid therapy.
4. Use of any investigational or non-registered product (drug or vaccine) within the past 30 days before study start and during study.
5. Psychiatric or addictive disorders of the patient that may have compromised his/her ability to give informed consent or to comply with the trial procedures.
6. Significant dental/oral disease, including prior history or current evidence of osteonecrosis/osteomyelitis of the jaw, active dental or jaw condition requiring oral surgery, non-healed dental/oral surgery, or planned invasive dental procedures for the course of the study.
7. Relevant immune deficiencies or relevant autoimmune diseases as assessed by the investigator.
8. Other malignancies within the past three years requiring treatment, except for basal or squamous skin carcinomas or carcinoma *in situ* of the cervix.
9. Serious cardiac, gastrointestinal, hepatic, or pulmonary disease.
10. Patients with serious intercurrent illness, requiring hospitalization.
11. Other serious illnesses, e.g., serious infections requiring intravenous antibiotics or bleeding disorders.

12. Hypersensitivity to the active substances of study treatment or to any of the excipients (calcium, vitamin D).
13. Hereditary fructose intolerance.
14. Women of childbearing potential: Refusal or inability to use effective means of contraception.
15. Female patients: The patient was pregnant or lactating or planning to become pregnant within 5 months after the end of the treatment.

Investigational medicinal product (dosage, method of administration, batch number): SoC treatment with nivolumab (alone or in combination with ipilimumab) or pembrolizumab and denosumab – according to the respective Summary of medicinal Product Characteristics (SmPC). Therefore, the batch or lot numbers of the administered drugs were not collected during this study.

Duration of treatment: The total treatment duration was not defined, as this study aimed to generate translational data. Treatment with PD-1 inhibitor and denosumab was at the discretion of the treating physician and could be performed beyond the participation of a patient in the study as per SoC.

Reference product (dosage, method of administration, batch number):

Not applicable.

First reference drug: Not applicable.
Second reference drug: Not applicable.

Unblinding: Not applicable.

Efficacy evaluation: Blood samples to assess the primary endpoints were planned to be taken at baseline (0-28 days prior to treatment initiation) and after 4 weeks, 12 weeks, and 24 weeks of therapy. The ORR was planned to be determined at 12 weeks and 24 weeks after therapy start by RECIST 1.1[1] criteria. Response rate of bone metastases was planned to be determined by RECIST 1.1[1] and by bone scans after 12 weeks and 24 weeks of therapy[2].

Safety evaluation: All adverse events (AEs), including serious adverse events (SAEs) and clinically significant laboratory abnormalities (ones that required medical or surgical intervention or lead to investigational medicinal product (IMP) interruption, modification, or discontinuation), were assessed and documented according to NCI CTCAE version 5.0. AEs were documented starting from the date of the first study drug administration until day 169 or 30 days after premature therapy discontinuation, whichever occurred first. SAEs were documented starting from the date of the signed informed consent until end of study of the respective patient or 30 days after study drug discontinuation, whichever occurred first.

Statistical methods: The primary objective of this trial was to evaluate immunological and biological effects of the combination of PD-1 and RANKL inhibition. Immunological effects of therapy were planned to be determined by comparison of baseline results and on treatment results by Bayesian inference. The profile over time as well as the time point with the highest difference to baseline were planned to be assessed. However, due to the low number of evaluable samples, the respective values were analyzed descriptively stratified by the achievement of disease control per patient. Levels of T-cell subsets as well as cytokine and chemokine levels were assessed over time.
In addition to the data sets defined in the protocol (Intention to Treat [ITT] and Per Protocol [PP] set), the modified Per Protocol (mPP) set was also analyzed, which is defined as all subjects who received at least two administrations of ICI and denosumab.

Summary of results:
Patient characteristics: Of the 17 patients included in this study, 52.9 % were male and 47.1 % were female. The median age at registration was 52 years (range: 34 – 78 years).

The ECOG performance status of most patients (88.2 %) was grade 0 at baseline. 16 patients received a combination of nivolumab and ipilimumab, 1 patient received pembrolizumab, and all patients received denosumab.

Efficacy: The response rate of bone metastases[2] and the ORR were determined for different datasets.

Population	Response rate of bone metastases				Overall response rate				
	Number of patients[1]	Number of patients with response	Rate	95% CI	Time point	Number of patients[2]	Number of patients with response	Rate	95% CI
ITT	14	9	64.29	35.14- 87.24	Week 12	16	2	12.50	1.55- 38.35
					Week 24	14	2	14.29	1.78- 42.81
mPP	12	8	66.67	34.89- 90.08	Week 12	14	1	7.14	0.18- 33.87
					Week 24	12	1	8.33	0.21- 38.48
PP	8	7	87.50	47.35- 99.68	Week 12	8	0	0	0.00- 36.94
					Week 24	8	1	12.50	0.32- 52.65

1 Patients without a post-baseline assessment where not included in the calculation of rates.
2 Patients without an assessment at a given time point were not included in the calculation of rates.

Tolerability: During the study, a total of 277 AEs occurred with each of the 17 patients having at least one AE. For 15 patients at least one AE of grade ≥3 was reported. In total, 25 SAEs occurred in 13 patients. For 11 patients, at least one SAE of grade ≥3 was reported. No Suspected Unexpected Serious Adverse Reactions (SUSARs) or grade 3/4 infusion related reactions were reported.
The most frequently reported categories of AEs were general disorders and administration site conditions (82.4 % of patients), investigations (76.5 %), and gastrointestinal disorders (64.7 %). A causal relationship between AEs and the study treatment was reported for nivolumab (146 AEs), ipilimumab (144 AEs) and pembrolizumab (5 AEs), while causality was more rarely reported for denosumab (35 AEs). A causal relationship to both nivolumab and ipilimumab treatment was assigned for 13 of the 25 reported SAEs. No SAEs were reported to have a causal relationship to denosumab or pembrolizumab treatment.
For 9 of the 17 patients treated, at least one treatment interruption (temporary or permanent) due to AEs was documented in the eCRF. For 7 of these patients, treatment was permanently discontinued because of AEs.
One patient died due to an AE (immune related tubulointerstitial nephritis), and a causal relationship to nivolumab and ipilimumab was reported.

Translational Research: The translational research program within this study focused on the detection of surrogate and/or predictive biomarkers for a successful immune therapy of melanoma based on the concurrent administration of ICI and denosumab.
The expression of the immune modulatory molecules RANK and RANKL on tumor tissue was determined via immunohistochemistry (IHC). Tumor tissue samples of 17 patients were analyzed, but for 1 patient, the sample quantity was not sufficient for an evaluation, and for another patient, the histo-score (H-score) of RANKL was not evaluable. Unexpectedly, virtually no positive signals were detected, neither on tumor cells nor on tumor-associated inflammation: For 1 patient, all H-scores determined for RANK were 5, while the H-score for RANKL was 10. For another patient, all H-scores for RANK were 0, and the H-score for RANKL was 2. For all other patients, H-scores for both stainings were 0.

Patients were classified according to response to treatment. Dynamic changes in T-cell subsets (naïve, central memory, activated and effector T-cells) and serum chemokines and cytokines over time were evaluated by flowcytometry and multiplexed assays. No differences in the T-cell dynamics were found between patients with or without achievement of disease control. Regarding serum parameters, a difference was only observed in the temporal course of levels of IL-27.

Conclusions: No clear association of the dynamic changes of T-cell subsets and most of the blood cytokines and chemokines analyzed and the clinical outcome (i.e., disease control or progressive disease) could be determined.
Regarding the evaluation of bone metastases, differentiation between response and stable disease was difficult, as imaging techniques such as PET-CT were not consistently applied. Therefore, also patients with an apparent stable disease were counted as having response regarding bone metastases. Due to this fact, the response rates of bone metastases appeared promising, but the ORR was unexpectedly low. However, the patient cohort studied appeared to have a high need of intense treatment and a rather poor prognosis.
It should be noted that the number of patients and especially the availability of evaluable samples were low, making it rather difficult to draw clear conclusions. Moreover, the numbers of evaluable samples varied between the examined time points. As only 2 patients received the study treatment for the complete period of 24 weeks, there was a strong decrease of available data towards the end of the treatment period. Moreover, the analysis included patients that had already discontinued treatment or have had treatment interruptions in the course of the study.
Besides, the results are difficult to interpret in the absence of a control group without denosumab treatment. Therefore, it would be interesting to perform a separate analysis of T-cell subsets and serum parameters identical to the methods of the present study in patients treated with the combination of nivolumab and ipilimumab without denosumab to be able to compare those with the presented results.

Date of report: October 27th, 2022

Protocol Versions and Amendments

Version	Date of Protocol	Date of Submission	Comments
Version 1.0	11.04.2018	22.08.2018	Final version for competent authorities and ethics committee
Version 2.0	25.06.2019	04.09.2019	In a first amendment of the study protocol, endpoints were widened to dynamic changes of cells, inclusion and exclusion criteria were adapted to more common medical standards for the addressed indication (e.g., the planned therapy with PD-1 blocking antibodies was opened by including the combination of nivolumab and ipilimumab), the number of clinic visits was adapted to the treatment schedules, and it was clarified that the study represents a hypotheses-generating approach as there was no pre-specified hypothesis.