Prospective evaluation of immunological, molecular-genetic, image-based and microbial analyses to characterize tumor response and control in patients with unresectable stage III NSCLC treated with concurrent chemoradiotherapy followed by consolidation therapy with durvalumab (PRECISION): protocol for a prospective longitudinal biomarker study

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Background: Concurrent platinum-based chemoradiotherapy (CRT) followed by durvalumab maintenance treatment represents the new standard of care in unresectable stage III non-small cell lung cancer (NSCLC). In this prospective hypothesis-generating single-center study, we aim to identify a framework of prognostic and predictive biomarkers by longitudinal characterization of tumor- and patient (host)-related parameters over all phases of multimodal treatment.

Methods: This study will enroll 40 patients (≥18 years, Eastern Cooperative Oncology Group performance status (ECOG PS) 0–2, with a diagnosis of PD-L1 positive (≥1%), inoperable stage III NSCLC) with an indication for CRT followed by maintenance treatment with durvalumab according to European Medicines Agency (EMA) approval. Comprehensive analysis will include peripheral blood cellular and humoral immunophenotyping and circulating tumor DNA as well as gut/saliva microbiota analyses. Additional morphological analysis with ^{18}F-FDG-PET/computed tomography (CT) before, 6 weeks, 6 and 12 months after the end of CRT is included. Statistical analysis using multiple testing will be used to examine the impact of different parameters on progression-free survival (PFS) and overall survival (OS) as well as tumor response and response duration.

Discussion: This protocol describes the methodology of a comprehensive biomarker study in order to identify a framework of prognostic and predictive markers for unresectable stage III NSCLC in a real-world setting.

Introduction

In PD-L1 positive (≥1%), unresectable stage III non-small cell lung cancer (NSCLC), concurrent platinum-based chemoradiotherapy (CRT) followed by maintenance treatment with the PD-L1 inhibitor durvalumab represents the new standard of care with approval of the European Medicines Agency (EMA) based on the promising results of the PACIFIC study (1-6). However, prognostic and predictive markers for this novel tri-modal treatment approach remain unclear. Historically, aspects such as patients' general condition, pulmonary function as well as tumor volume, extent of the tumor invasion and lymph node involvement have been of crucial importance for prognosis and treatment efficacy (7-9). Due to the introduction of immune check-point inhibition in contemporary cancer treatment, the state of the host's immunity and microbiome has become more relevant for treatment efficacy and prognosis (10-12).

In order to identify a framework of prognostic and predictive biomarkers in unresectable stage III NSCLC, we will perform a non-interventional explorative biomarker study with longitudinal comprehensive characterization of the patient (host) and tumor as well as their longitudinal changes during the course of tri-modal therapy. This study will provide invaluable information on the immunological, molecular-genetic, morphological and microbial parameters in patients with unresectable stage III NSCLC treated with durvalumab maintenance for 12 months after concurrent CRT. The following article was written in accordance with the SPIRIT reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-21-1010/rc).

Methods

The study protocol and informed consent documents were approved by the ethics committee of the Ludwig-Maximilians-University of Munich (approval number: 20-0502), and informed consent will be obtained from all patients. All procedures performed in this study will be in accordance with the Declaration of Helsinki (as revised in 2013) and adhere to Good Clinical Practice guidelines.

Study design and patients

This non-interventional hypothesis-generating single-center biomarker study aims at a longitudinal comprehensive phenotyping of the patient (host) and the tumor regarding molecular-genetic features, immunological, morphological, image-based and microbial characteristics with additional assessment of changes during tri-modal treatment and potentially recurrent disease. We will enroll 40 patients with unresectable stage III NSCLC who will receive concurrent platinum-based CRT followed by maintenance therapy with the PD-L1 inhibitor durvalumab at the Department of Radiation Oncology, University Hospital Munich (Ludwig-Maximilians-University; LMU).

All patients will be discussed at the thoracic oncology tumor board of the University Hospital Munich (LMU) and will be screened for eligibility in the study regarding histopathological and radiological findings and patients' characteristics.

Key inclusion and exclusion criteria
❖   Inclusion criteria:
- Patient age ≥18 years;
- Histologically/cytologically confirmed diagnosis of NSCLC;
- Patients with unresectable NSCLC and tumor stage III A–C according to the Union Internationale Contre le Cancer (UICC) TNM 8th edition;
- Eligible for concurrent platinum-based CRT followed by durvalumab maintenance treatment according to the latest approval in Germany;
- No invasive malignancy (mesenchymal, hematological or epithelial) in the five preceding years;
- Eastern Cooperative Oncology Group (ECOG) performance status 0–2;
- Lung function parameters (before or after

bronchodilation): FEV1 ≥1.0 L and/or DLCO-SB ≥40% predicted;

- ◆ A maximum of two cycles of induction chemotherapy are permissible before the start of CRT.
- ❖ Exclusion criteria:
  - ◆ Simultaneous participation in another clinical trial;
  - ◆ Mixed histology of small-cell lung cancer and NSCLC;
  - ◆ Brain metastases confirmed by a contrast enhanced cranial MRI;
  - ◆ Prior receipt of immunotherapy or an investigational medicinal product;
  - ◆ Previous exposure to an anti-PD-1 or anti-PD-L1 antibody;
  - ◆ Pneumonitis ≥ grade 2 as a result of prior CRT;
  - ◆ Patients with a non-active disease in the last 5 years can be included, but only after consultation with the responsible investigator of the study or his representative;
  - ◆ Primary immunodeficiencies in previous history
  - ◆ Prior interstitial lung disease (ILD);
  - ◆ Prior autoimmune disease;
  - ◆ Previous organ transplantation with subsequent therapeutic immunosuppression.

After consent and enrollment, blood samples will be collected during clinical routine 5–10 days before the start of CRT and subjected to immunophenotyping of peripheral blood mono-nuclear cells (PBMC), to genetic testing using the Oncomine Pan-Cancer Cell free Assay (Life Technologies, Carlsbad, CA, USA) next generation sequencing (NGS) as well as the detection of mutations in short circulating tumor total nucleic acid (ctTNA: ctDNA and ctRNA). At the same time of blood sampling, saliva and stool sample for microbiome analyses will be collected.

During treatment, a comprehensive characterization including immunophenotyping of PBMC, ctDNA as well as gut/saliva microbiome analyses will be performed.

Immunophenotyping will be performed using a set of eligible markers (CD3, CD4, CD8, CD19, CD20, CD14, CD16, slan, HLA-DR) to quantify the major blood cell lineages like T-cells, B-cells, NK-cells, and monocytes including their subpopulations in absolute numbers and frequencies. Further marker panels (e.g., FoxP3, Ki67, CD25, CX3CR1, CD38) are used to assess immunocyte subpopulations like Tnaive, Treg, Teff, Tmem, Texh that are relevant in immune response, regulation, and killing.

Bacterial assemblages will be analyzed using high-throughput sequencing based on 16S rRNA profiles to selectively identify the transcriptionally active bacteria. RNA from saliva and faecal samples will be extracted using the RNeasy kit (Qiagen, Mississauga, ON, Canada). After DNA digestion, first-strand cDNA will be synthesized using Superscript IV First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA) and random primers. Amplicon libraries will be generated as described before in detail (13,14).

Sample collection will be repeated mid-treatment (at the 15 fraction mark), at the end of CRT and 3, 6 and 12 months after the end of thoracic irradiation (see *Figure 1*).

18F-FDG-PET/computed tomography (CT) will be performed 5–10 days before start of radiotherapy (RT), 6 weeks, 6, 12 and 24 months after the end of CRT. Patients with asymptomatic disease progression will be assessed according to our internal standard operating procedure (SOP) with CT of the chest and abdomen every 3 months and every 6 months an MRI of the brain. In patients with symptomatic disease, imaging modality will depend on physician's choice followed by the internal SOP.

Lung function will be assessed before the start of, at the end and 6 weeks as well as 3, 6 and 12, 18, 24 months after CRT.

Histopathological examination and NGS will be performed at the accredited Institute of Pathology (Faculty of Medicine, LMU of Munich, Munich, Germany).

Follow-up until 5 years after the end of chemoradiation will be performed by the Department of Radiation Oncology at the University Hospital Munich (LMU) according to the clinical SOPs as well as treatment-related side effects according to Common Terminology Criteria for Adverse Events (CTCAE) version 5.

Study objectives

The primary objective is to identify early immunological and morphological biomarkers and their dynamic changes to predict progression-free survival (PFS) at 12 and 24 months [according to the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1, and PERCIST as assessed by means of blinded independent central review]. PFS is defined as the time from end of CRT to the date of the first documented event of tumor progression or death in the absence of disease progression.

The secondary objective is to identify predictive biomarkers for PFS at 6 and 18 months after CRT. PFS will be assessed by the investigators according to RECIST 1.1 and PERCIST. Overall response rate, local and distant tumor control (response duration) at 6 weeks, 6, 12, 18 and
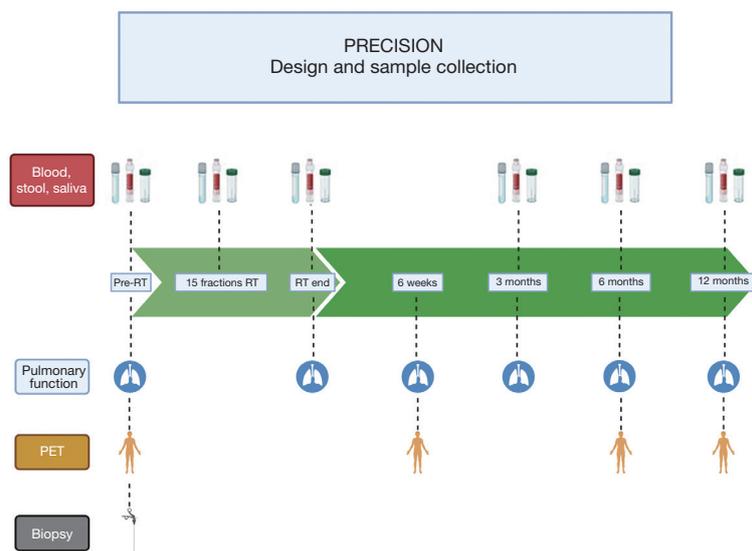
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Figure 1 Study design. This figure has been reproduced from Käsmann *et al.* (15) and written permission from the copyright holder (Springer Nature) has been obtained in order to reproduce the material. RT, radiotherapy; PET, positron emission tomography with 2-deoxy-2-[fluorine-18] fluoro-D-glucose integrated with computed tomography.

24 months from the end of CRT according to PERCIST and RECIST criteria will be correlated with potential biomarkers.

Statistical analysis

In this prospective explorative study, 40 patients will be enrolled between December 2020 and September 2022 based on previous single-center experience and published historical data:

- ❖ 20 patients will be progression-free at 24 months after the start of CRT (50% PFS rate);
- ❖ 12 patients will develop multiple distant metastases, including brain (30% rate);
- ❖ 3 patients will develop a single metachronous distant metastasis, including brain (7.5% rate);
- ❖ 10 patients will develop a loco-regional recurrence (25% rate) at 24 months after completion of CRT.

Five cancer-related deaths are anticipated until end of follow-up period (24 months after start of CRT). A 5% drop-out rate was estimated.

With a sample size of 40 patients, an explorative biomarker study using multiple testing is justified to evaluate predictive and prognostic biomarker by the scarcity of quality samples, capacity and costs of molecular techniques. A further confirmation step is planned in an external validation cohort.

Discussion

Implementation of maintenance treatment with the PD-L1 inhibitor durvalumab in unresectable stage III NSCLC with at least stable disease after completion of concurrent CRT has led to a marked improvement in patient prognosis and established a new standard approach for this heterogeneous disease. According to the PACIFIC trial and reported real-world data, this novel tri-modal approach has led to a significant increase in local-regional control, PFS and overall survival (OS) (5,14).

Despite the unprecedented historical improvement of long-term outcome, a significant number of patients will develop recurrent disease. Importantly, there is a paucity of data regarding salvage treatment options. Comprehensive analysis of recurrence predictors, including clinical factors and biomarker candidates are pertinent.

Additionally, further intensification of this tri-modal approach with application of immune checkpoint inhibition and CRT simultaneously followed by the established maintenance treatment is also the subject of several ongoing studies (15,16).

In the face of the achieved progress in this field, underlying mechanisms explaining the durable positive effect of durvalumab maintenance treatment on local and distant tumor control after CRT are still unclear (3,17,18). Hence, the ongoing trials should be focused on deciphering

these synergistic effects.

PRECISION is a single-center explorative biomarker study designed to generate a framework of specific tumor- and patient (host)-associated parameters with significant impact on the processes of tumor clearance during CRT and establishment of long-lasting antitumor immune response during the durvalumab maintenance phase of multimodal treatment.

The strength of this study is a comprehensive and longitudinal biological material collection and analysis. Analyses will be performed at six time points: three time points during CRT and three during the maintenance treatment phase, respectively. This study will also evaluate the dynamic changes of the tumor- and patient (host)-related parameters across the treatment time. The association of the detected cfDNA/ctDNA levels/variant frequencies with objective tumor response based on the RECIST and PERCIST criteria and pattern of failure (loco-regional *vs.* distant) may add new relevant data for patient follow-up. Correlation of the dynamic changes of PBMCs with acute and subacute treatment-related toxicity, especially pneumonitis, should provide crucial knowledge for future studies to mitigate adverse effects.

Previous studies found a mutual relationship between the gut microbiota and administered immunotherapy especially in malignant melanoma patients (19,20). Despite an unknown underlying mechanism, gut microbes may modulate adaptive immunity, either in a beneficial or harmful manner and might influence the efficacy and toxicity of immunotherapy via the microbiota and modulation of adaptive immunity. Our study contributes to a further understanding of the patient immune system and identify potential biomarkers. In order to confirm our results, a validation preferably in an external cohort is warranted and planned.

In summary, PRECISION will provide invaluable information regarding patient (host) systemic immune landscape and the cancer—patient (host) immune interplay at different phases of tri-modal treatment. The key challenge will be defining a specific framework of immunological, molecular-genetic and microbial parameters and their dynamic changes with specific impact on long-term tumor control and patient prognosis.

Acknowledgments

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This biomarker study will be conducted in accordance with the Declaration of Helsinki (as revised in 2013) and adhere to Good Clinical Practice guidelines. Prior to the initiation of the study, local Ethics Committee/Institutional review board approval (approval number: 20-0502) has been given, and informed consent will be obtained from all patients.

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