Clinical Study Protocol Nostra:Biome - Oncology

Title: *A Prospective, Interventional Study Investigating the Impact of Personalized Microbiome Modulation on Treatment Outcomes in Patients with Melanoma and Non-Small Cell Lung Cancer (NSCLC) Receiving Pembrolizumab*

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Objective

This study aims to evaluate the effectiveness of personalized microbiome modulation, guided by an AI-based solution, in improving the treatment outcomes to pembrolizumab monotherapy in patients with advanced melanoma and NSCLC. The primary hypothesis is that identifying unique microbiome signatures and determining the immune load from the microbiome, followed by personalized modifications of the microbiome, will enhance the overall response rate (ORR) and progression-free survival (PFS) compared to historical benchmarks of pembrolizumab therapy alone.

Background and Rationale

Several recent trials have focused on how gut microbiota might affect the efficacy and toxicity of immune checkpoint inhibitors (ICI), like pembrolizumab. One key area of interest is the microbiota's ability to modulate immune responses by enhancing or suppressing them, depending on the microbial composition. A study conducted by Gunjur et al and published in Nature Medicine (March 2024) highlighted that a signature collection of microorganisms in an individual's gut bacteria may help identify those who would benefit from combined ICI, emphasizing the importance of understanding the microbiome at the strain-level, not just species-level, opening a new way towards personalized medicine. Furthermore, this signature was used to train a machine learning model to predict who would benefit from combination immunotherapy. Additionally, the authors extrapolated and demonstrated similar outcomes when using the microbial signature through a meta-analysis of previous studies that used combined ICB on patients diagnosed with melanoma, from different geographic locations. Interestingly, distinct performance was observed when only anti-PD1 was administered, suggesting that future analyses must be done in a more personalized fashion regarding the relationship between the gut microbiome and the ICI used.

Pembrolizumab, an anti-PD-1 antibody, is a standard treatment for advanced melanoma and NSCLC, particularly in tumors expressing PD-L1. While effective in a subset of patients, response rates remain suboptimal for many. Personalized microbiome modulation offers a novel therapeutic approach to improve these outcomes by identifying and targeting individual microbiome signatures.

Study Design
Type: Interventional, Open-label, Multicentric
Model: Parallel Assignment
Purpose: To evaluate the impact of microbiome modulation, guided by an AI-based solution, on the clinical efficacy of pembrolizumab
Enrollment: 100 patients
Study Duration: 2 years (with follow-up for up to 6 years)
Study Locations: Multicenter study across 7 clinical sites in Romania (Alba-Iulia, Bucharest, Cluj, Constanta, Craiova, Iasi, and Timisoara)

Study Population
Participants are adults diagnosed with either unresectable or metastatic melanoma or NSCLC. All participants will receive pembrolizumab monotherapy as the standard of care. Participants will undergo microbiome analysis and personalized modulation as part of the investigational intervention.

- Sample Size: 100 patients (50 melanoma and 50 NSCLC)
- Cohort 1: Patients with advanced unresectable/metastatic melanoma
- Cohort 2: Patients with metastatic NSCLC, PD-L1 with TPS ≥50%

Inclusion Criteria
- Adults (≥18 years) with histologically confirmed advanced melanoma or NSCLC.
- ECOG performance status of 0-2.
- Eligible to receive pembrolizumab as monotherapy.
- Willingness to provide stool, blood, and tumor tissue samples.
- Able to comply with study procedures, including sample collections and visits.

Exclusion Criteria
- Active central nervous system metastases.
- Uncontrolled severe autoimmune diseases or infections.
- Recent (within 6 months) significant cardiovascular events.
- Concurrent malignancies requiring treatment.
- Systemic corticosteroid use (>10 mg of prednisolone daily).

Intervention/Treatment
All patients will receive pembrolizumab at standard dosing for their respective cancers:

- Pembrolizumab (for melanoma and NSCLC): Administered every 3 weeks, up to 2 years or until disease progression or unacceptable toxicity.

In addition, patients will undergo microbiome assessment through stool sample collection at baseline, during treatment (every 6 weeks), and upon disease progression or completion of the study. Using a proprietary AI-based platform, the microbiome signatures will be analyzed, and personalized dietary, probiotic, or prebiotic interventions will be designed to modulate the gut microbiome. These interventions are aimed at reducing pro-inflammatory signatures and optimizing immune response to the pembrolizumab treatment.

Study Measures
Primary Outcome
- Progression-free survival (PFS) of 1 year.

Secondary Outcomes
- Overall Response Rate (ORR): Assessed via RECIST 1.1 at regular intervals.
- Overall Survival (OS): Follow-up for up to 6 years.
- Microbiome-Immune Correlation: Investigating the relationship between microbiome characteristics and treatment efficacy.
- Immune-Related Adverse Events (irAEs): Correlation of microbiome signatures with the incidence and severity of irAEs.
- Biological Sample Repository: Building a library of biospecimens (blood, stool, and tumor tissue) for future research on microbiome and immune modulation.

Biospecimen Retention
- Blood samples, stool samples, and tumor biopsies will be collected and stored for future analysis. Tumor and organ samples may be obtained following treatment-related toxicity when available.

Statistical Analysis

The study aims to compare the response rates (PFS, ORR, and OS) of pembrolizumab-treated patients undergoing microbiome modulation with historical benchmarks of pembrolizumab monotherapy. Descriptive and inferential statistical methods will be employed to assess the added benefit of microbiome modulation.

- Sample Size Justification: Based on prior studies, it is estimated that personalized microbiome modulation could improve ORR by 10-20% compared to the 30-40% historical ORR for pembrolizumab alone.
- Multivariate Analysis: Subgroup analyses will be conducted to assess the differential impact of microbiome modulation on melanoma versus NSCLC and identify biomarkers of response.

Ethics and Safety Considerations

The study will adhere to ICH-GCP guidelines. All patients will provide informed consent before participation. The study protocol will be reviewed and approved by local ethics committees. An independent data monitoring committee (DMC) will be established to ensure participant safety and study integrity.

Collaborators and Investigators

Sponsor: To Be Determined (TBD).
Collaborators: TBD.
Principal Investigators: TBD at each of the participating centers.

Conclusion

This study represents a novel approach by integrating personalized microbiome modulation with pembrolizumab therapy in patients with advanced melanoma and NSCLC. The results could provide evidence for a new strategy to enhance the efficacy of immune checkpoint inhibitors by leveraging the gut microbiome's influence on systemic immunity.

Study SCHEME

Study Schema: Interventional Study on Microbiome Modulation and Pembrolizumab Therapy in Melanoma and NSCLC

| **Patient Identification** |
Patients diagnosed with advanced melanoma or NSCLC, who are about to commence systemic therapy with pembrolizumab (anti-PD1).

| **Registration to Relevant Cohort** |

- Signed written informed consent.
- Eligibility check based on inclusion/exclusion criteria (e.g., histological confirmation, ECOG status 0-2, unresectable or metastatic disease).

| **Baseline Sampling and Microbiome Interpretation** |

- Collection of minimum dataset: clinical information, blood, and stool samples.
- AI-based analysis of stool samples to identify unique inflammatory microbiome signatures and immune load.
- Personalized recommendations are provided for microbiome modulation (e.g., dietary changes, probiotics, prebiotics).

| **3 Months: Data Collection** |

- Collection of blood and stool samples for microbiome monitoring.
- Imaging studies and clinical data collection for response evaluation.
- Adjust microbiome modulation plan based on AI-driven insights from stool analysis.

| **6 Months: Data Collection** |

- Re-assessment with blood and stool samples.
- Imaging and clinical assessment.
- Continued microbiome modulation based on updated findings.

| **9 Months: Data Collection** |

- Further blood and stool samples.
- Imaging and clinical assessment.
- Adjustment of microbiome modulation as necessary.

| **At 1 Year or Treatment Stopping** |

- Full data collection including blood, stool, and imaging.

- Treatment discontinuation if disease progression or unacceptable toxicity occurs.

| **Main Study Objectives** |

To determine if personalized microbiome modulation improves efficacy of checkpoint inhibitor therapy.
Primary Outcome: Efficacy.

Secondary Outcomes: Toxicity.