Clinical Study Protocol: Interventional Study on Microbiome Modulation in Patients Receiving Nivolumab + Ipilimumab Therapy

Study Title:
The Influence of the Intestinal Microbiota on the Efficacy and Toxicities of Nivolumab + Ipilimumab Combination Therapy in Patients with Melanoma and Renal Cancer: A Prospective, Interventional Study on Microbiome Modulation

Background and Rationale

Immune checkpoint inhibitors (ICIs) like nivolumab (anti-PD-1) and ipilimumab (anti-CTLA-4) have transformed the treatment of multiple cancers, including melanoma and renal cell carcinoma. However, the overall response rate (ORR) remains suboptimal, with only about 50-60% of patients responding to combination immune checkpoint blockade (CICB), and a significant portion experiencing grade 3-4 immune-related adverse events (irAEs). Recent studies highlight the gut microbiome's role in influencing the efficacy and toxicity of ICIs. Microbiome signatures have shown promise as predictive biomarkers for cancer immunotherapy outcomes.

This study aims to assess the impact of personalized microbiome modulation in improving the response rate and reducing toxicity in patients receiving nivolumab + ipilimumab. AI-driven microbiome interpretation will guide the personalized interventions, which will include dietary adjustments and probiotics/prebiotics designed to optimize the intestinal microbiome for enhanced immune response and improved therapy outcomes.

Study Objectives

Primary Objective:
- To determine whether personalized microbiome modulation can improve the overall response rate (ORR) of nivolumab + ipilimumab combination therapy in patients with melanoma and renal cancer.

Secondary Objectives:
- Evaluate progression-free survival (PFS) at 1 year.
- Assess overall survival (OS) up to 6 years.
- Correlate microbiome signatures with the incidence and characteristics of immune-related adverse events (irAEs).
- Build a repository of biological samples for future research on microbiome-immune interaction in cancer immunotherapy.

Study Design

Type:
Interventional, Open-label, Multicenter (7 locations in Romania: Alba-Iulia, Bucharest, Cluj, Constanta, Craiova, Iasi, Timisoara)

Interventions:
- Cohort 1 (Melanoma): Unresectable AJCC Stage 3/4 melanoma, treated with induction nivolumab + ipilimumab followed by maintenance nivolumab.
- Cohort 2 (Renal Cancer): Advanced renal cell carcinoma treated with nivolumab + ipilimumab combination.

Microbiome Modulation:
Patients will undergo AI-based microbiome analysis at baseline and at regular intervals (every 3 months). Personalized microbiome modulation strategies, including dietary changes, probiotics, or prebiotics, will be provided based on individual microbiome signatures.

Sample Size:
Minimum 100 patients (50 with advanced melanoma and 50 with advanced renal cell carcinoma)

Eligibility Criteria

Inclusion Criteria:
- Signed informed consent.
- Age ≥18 years.
- Histologically confirmed unresectable melanoma (AJCC Stage 3/4) or renal cell carcinoma.
- ECOG performance status of 0–1.
- Eligible for nivolumab + ipilimumab combination therapy.
- Measurable disease by RECIST v1.1.
- Willingness to comply with study procedures, including sample collection.

Exclusion Criteria:
- Active central nervous system metastases.
- Severe autoimmune conditions, active infections, or significant medical conditions interfering with the study.
- Recent cardiovascular events (e.g., MI or stroke within the last 6 months).
- Previous treatment with CICB (anti-CTLA4 or anti-PD1).
- Systemic corticosteroid use >10 mg/day.
- Pregnant or lactating women.

Study Interventions and Schedule

| Step | Description |

| Patient Identification | Patients with melanoma or renal cell carcinoma scheduled for nivolumab + ipilimumab combination therapy. |

| Registration and Baseline Sampling | - Signed informed consent.
- Collection of baseline stool, blood, and clinical data.
- Baseline microbiome analysis using AI-driven tools. |

| Microbiome Interpretation and Modulation | - AI analysis to identify individual microbiome signatures and immune load.
- Personalized microbiome modulation plan (dietary, probiotic, and prebiotic interventions). |

| Nivolumab + Ipilimumab Therapy | - Nivolumab (3 mg/kg) + ipilimumab (1 mg/kg) every 3 weeks (four doses), followed by maintenance nivolumab (240 mg every 2 weeks or 480 mg every 4 weeks). |

| Monitoring and Adjustments | - Stool and blood samples collected at 3, 6, and 9 months.
- Regular imaging studies (every 3 months) to assess treatment response.
- Continuous microbiome modulation based on follow-up microbiome analyses. |

| Final Evaluation at 1 Year | Comprehensive assessment, including stool and blood sampling, imaging, and microbiome reanalysis. Therapy may stop earlier in case of disease progression or unacceptable toxicity. |

| Long-Term Follow-up | Patients will be followed for overall survival (OS) up to 6 years. |

Study Measures

Primary Outcome:
- Overall Response Rate (ORR) assessed using RECIST 1.1 and immune-related RECIST (iRECIST).

Secondary Outcomes:
- Progression-free survival (PFS) at 1 year.
- Overall Survival (OS) up to 6 years.
- Correlation between microbiome signatures and immune-related adverse events (irAEs).
- Correlation between baseline microbiome signatures and clinical efficacy of nivolumab + ipilimumab.
- Build a repository of biospecimens (stool, blood, tumor tissue) for future research.

Sample and Biospecimen Retention

Biospecimens:
- Stool and blood samples collected at baseline and every 3 months for microbiome analysis.
- Tumor biopsies and organ samples collected when available (in cases of severe irAEs).
- Samples retained for future microbiome research.

Timeline and Study Flowchart

| *Study Timeline* | *Events* |

| Month 0 (Baseline) | Patient registration, informed consent, baseline stool and blood sampling, AI-driven microbiome analysis, initiation of nivolumab + ipilimumab therapy, personalized microbiome modulation recommendations. |

| Month 3 | Data collection (stool, blood, imaging), microbiome re-analysis, adjustment of microbiome modulation strategy. |

| Month 6 | Continued data collection (stool, blood, imaging), further microbiome modulation as required based on response. |

| Month 9 | Data collection (stool, blood, imaging), final adjustments to microbiome modulation. |

| Month 12 (Final Evaluation) | Comprehensive evaluation, including stool and blood sampling, imaging, and final microbiome analysis. Therapy discontinuation if necessary.

| Long-Term Follow-Up | Collection of OS and irAE data up to 6 years. |

Study Schema

| *Step* | *Description* |

| Patient Identification | Melanoma or renal cancer patients eligible for nivolumab + ipilimumab. |

| Baseline Sampling | Baseline collection of stool and blood samples for AI-driven microbiome analysis. |

| Microbiome Modulation | Personalized modulation based on microbiome profile (dietary, probiotics, prebiotics). |

| Combination Therapy | Nivolumab + ipilimumab induction, followed by nivolumab maintenance.|

| Monitoring (3, 6, 9, 12 months) | Data collection, microbiome reassessment, imaging, and modulation adjustments. |

| Final Evaluation | Comprehensive assessment and therapy stopping if disease progression or unacceptable toxicity occurs. |
| Long-Term Follow-Up | OS and toxicity data collected up to 6 years. |

This interventional study design focuses on evaluating the impact of personalized microbiome modulation on the efficacy and safety of nivolumab + ipilimumab combination therapy, with the ultimate goal of improving response rates and reducing toxicities in patients with melanoma and renal cancer.