ImmunoTools *FlowISiAM* Award 2026

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Identification of biomarkers of primary and secondary resistance in melanoma patients treated with immune checkpoint inhibitors

Background

Melanoma is a highly aggressive cancer that originates mainly from melanocytes in the skin, though it can also develop in other tissues, including mucosal surfaces and the eye uveal tract **[1].** Effective systemic treatments for melanoma patients include immune checkpoint inhibitors (ICIs), such as anti-programmed death (PD)-1 and anti-cytotoxic T-lymphocyte antigen (CTLA)-4 antibodies. ICIs have transformed the treatment of patients with early- and late-stage melanoma, improving clinical and radiological responses and extending overall survival (OS) **[2]**. Nevertheless, resistance to ICIs is common. Approximately 55% of melanoma patients show primary resistance to PD-1 monotherapy, and 40% to the CTLA-4+PD-1 inhibitor combination. Furthermore, nearly 25% of patients who respond to treatment with a PD-1 inhibitor acquire resistance within two years **[3,4]**.

The presence of mutations in the *NRAS* gene appears to be a negative prognostic marker in melanoma. However, its role as a predictor of response to immunotherapy is unclear due to conflicting data in the literature **[5-8]**. NRAS mutations occur in 20% of melanomas **[9]**. The majority (>80%) involve a point mutation that substitutes a glutamine for a leucine at position 61. This mutation impairs protein activity and maintains RAS in its active state, resulting in the upregulation of the mitogen-activated protein kinase (MAPK) pathway and unopposed downstream signaling, which leads to uncontrolled cell growth, motility, and survival. Indeed, NRAS mutations in metastatic melanoma have been associated with more aggressive disease characteristics and shorter OS **[10-12]**. In our experience with patients treated with ICIs in the

adjuvant setting we found differences in OS between NRAS wild-type and mutated patients (manuscript submitted).

There are no predictive biomarkers that can identify which melanoma patients will develop resistance to ICIs and this project aims at investigating the immunological landscape of melanoma patients treated with ICIs to identify such biomarkers. Due to the uncertain benefits of ICIs for patients with NRAS mutations in both the adjuvant and metastatic settings, we will consider RAS-mutant, BRAF-mutant, and RAS/BRAF wild-type tumors.

It should be noted that ICIs are used not only for melanoma, but also for different tumor types, such as lung, breast, and colon cancers. The type and frequency of ICI-dependent immune-related adverse events (irAEs) are similar across all subpopulations, regardless of tumor type or disease stage. Identifying predictive biomarkers of response or resistance to ICIs will help avoid exposing patients to ineffective treatments and the onset of irAEs. Tumor immunotherapy landscape is now being reshaped by the advent of neoadjuvant therapy. However, evaluating the response to or resistance to ICIs in this setting is difficult, so developing effective biomarkers will be important.

Experimental design and expected outcomes:
We propose to analyze through *FlowISiAM* AT-test and ImmunoTools *AMANDEN* blood samples collected from melanoma patients undergoing ICI therapy, both in the adjuvant/neoadjuvant and metastatic setting. We will compare patterns in patients with NRAS-mutated melanomas to those with BRAF-mutated and BRAF/NRAS-wild-type tumors.

We already have a collection of whole blood, serum, plasma, and peripheral blood mononuclear cells (PBMC) from 100 melanoma patients at the beginning of therapy, during treatment, and at the time of progression. We would perform the *FlowISiAM* AT-test on these samples, analyzing DNAseX (Apo10) for aberrant apoptosis and TKTL1 for aberrant energy metabolism in circulating monocytes/macrophages. In fact, an alteration in the levels of these biomarkers could indicate resistance to ICIs and tumor recurrence before clinical evidence of the disease appears.

The ImmunoTools *AMANDEN* flow cytometry technique will be used to identify immune cell subtypes and activation status using different biomarkers, such as CD3, CD19, CD25, CD16, CD56, CD95, and CD45. This could lead to the identification of resistance-related epitopes, offering promising prospects for the future use of *FlowISiAM* in diagnosis and research. The results will be compared with the patient clinical response to ICIs and OS to identify patterns of response or resistance.

Biomarker identification will allow patients to avoid potentially harmful therapies and irAEs, anticipate biochemical evidence of disease progression limiting radiographic scans, and explore new therapeutic options that could prompt a specific response.

Key References:

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ImmunoTools *FlowISiAM* AWARD for

Rosa Falcone and **Paolo Marchetti** includes

antibodies for *FlowISiAM*, know how transfer and protocol, support regarding selection of specific antibodies against some specific biomarkers from INVIGATE, engagment regarding development of specific *AMANDEN*, and expert assistance in evaluating the results obtained, and integration into the ImmunoTools *FlowISiAM* network.

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