ImmunoTools *FlowISiAM* Award 2024

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Assessing Pathological Complete Response in Neoadjuvant Treatment of non-small cell lung cancer: A Non-Invasive Approach Using *FlowISiAM*.

Acronym: ANITA - **A**ssessment of **N**on-**I**nvasive **T**echniques for **A**chieving pCR in Lung Cancer

BACKGROUND AND RATIONALE

Lung cancer is the leading cause of cancer deaths worldwide [1], with non-small cell lung cancer (NSCLC) making up approximately 85% of all cases [2]. The advent of immunotherapy, particularly with immune checkpoint inhibitors (ICIs), has greatly improved the management of both locally advanced (stage III) and metastatic (stage IV) NSCLC, leading to better 5-year survival rates [3-5]. Consequently, ICIs have become the new standard of care, used either as a consolidation treatment after chemoradiotherapy for stage III NSCLC[6] or as the primary treatment for stage IV NSCLC without targetable oncogenic driver [7].

About half of all diagnosed NSCLC cases are at stage I-III [8]. While surgery is the primary treatment for early-stage lung cancer[9], only about one in four patients have a disease that can be surgically removed at diagnosis, and 30% to 55% of these cases recur after surgery [10, 11]. Recent clinical trials have shown the efficacy of immunotherapy as part of surgical strategies aiming for a cure, which may include neoadjuvant (before surgery), adjuvant (after surgery), or perioperative (spanning the period around surgery) administration of ICIs [12-

17]. Interestingly, both preclinical [18] and clinical [19] data suggest that neoadjuvant ICIs, which prime the body's immune response against tumor-specific antigens, are more effective in eradicating microscopic metastatic disease than adjuvant ICIs. This is particularly true when combined with platinum-based chemotherapy in early-stage NSCLC, resulting in better event-free and overall survival, and a marked increase in pathological complete response (pCR) compared to neoadjuvant platinum-based chemotherapy alone [20], emphasizing the potential of neoadjuvant ICI strategies to significantly improve patient outcomes. Moreover, neoadjuvant chemo-immunotherapy has redefined the boundaries of operable disease, with multi-station N2 disease now being considered for surgery, as shown in the NEOTORCH phase III and Phase II NADIM II trials [15, 21].

Importantly, approximately 25% of patients achieve a pathological complete response (pCR) in the neoadjuvant NSCLC setting[12-15, 21]. Moreover, achieving pCR in the context of neoadjuvant chemo-immunotherapy is a strong surrogate endpoint for overall survival (OS), as patients achieving pCR generally do not experience cancer recurrence (indicating better disease-free survival, or DFS) and tend to live longer (indicating better OS)[12, 14]. Unfortunately, patients with pCR face risks associated with surgery, including a mortality rate of 2-5%, morbidity of 16-21%, and the development of bronchopleural fistulas (0.5-1.8%) [22]. To date, no tests, including blood tests or imaging, have been able to distinguish between patients who achieve pCR and those who do not. Currently, surgery is the only method to differentiate these two populations.

Therefore, it is crucial to identify a non-invasive test that can detect pCR, thus potentially avoiding unnecessary surgery and reducing exposure to surgical risks. We aim to utilize a non-invasive tool, using a blood sample, called *FlowISiAM*, in the first cohort of patients with a known diagnosis of lung cancer, compared to patients without cancer, to establish the positive and negative predictive values, as well as to identify the false positive and false negative rates of the test.

In the second part of the test validation, we will apply *FlowISiAM* prospectively in a cohort of patients receiving neoadjuvant immune checkpoint inhibitors (ICIs) to differentiate between those who achieve pCR and those who do not. Lastly, we will also collect blood samples from patients at diagnosis and on the day of surgery to determine if *FlowISiAM* can serve as a non-invasive method to assess treatment efficacy and residual disease, in comparison to CT scan imaging, which remains the standard for evaluating treatment efficacy today.

Within this collaboration, ImmunoTools adjust the experimental and instrumental set-up for *FlowISiAM* analysis. INVIGATE will assist during the selection of optimized monoclonal antibodies for detection of NSCLC and intend to create proof-of-principle results for a joint research grant application.

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ImmunoTools *FlowISiAM* AWARD for

Pierre van der Bruggen and Frank Aboubakar Nana includes antibodies for *FlowISiAM*, know how transfer and protocol, support regarding selection of specific antibodies against specific biomarkers from INVIGATE, expert assistance in evaluating the results obtained, and integration into the ImmunoTools *FlowISiAM* network.