Next-Generation Immunotherapy: T Cell Function as a Predictive and Tuning Tool

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Background
Immune checkpoint inhibitors (ICIs), targeting the CTLA-4 and PD-L1 pathways, have revolutionized the treatment of advanced non-small cell lung cancer (NSCLC). Despite this progress, durable clinical responses are observed in only ~20% of patients, while approximately 30% experience rapid disease progression despite therapy (doi: 10.3390/cells11030320). ICI-based immunotherapy is currently the first-line treatment for patients with advanced NSCLC lacking targetable mutations or harboring KRAS mutations. Patient eligibility for ICI monotherapy or ICI-chemotherapy combinations is primarily guided by PD-L1 expression on tumor cells (doi: 10.1016/S0140-6736(24)01029-8). However, PD-L1 alone is an insufficient predictor of long-term outcomes. Alternative biomarkers-such as tumor mutational burden-have been investigated yet show inconsistent predictive value as they fail to capture the intrinsic immune competence, especially the functional capacity of T cells to respond upon immune reactivation (doi: 10.1016/j.annonc.2022.12.013).
The anti-tumor effect of ICIs relies predominantly on T cell infiltration into tumors and the reactivation of exhausted CD8+ T cells. Among various functional markers, the production of interferon-gamma (IFN-γ) by T cells strongly correlates with the efficacy of ICI-induced immune responses (doi: 10.1016/j.ccell.2020.08.005). Despite this, baseline assessment of peripheral T cell function-particularly IFN-γ production capacity following stimulation-is not routinely performed prior to ICI initiation, although preliminary studies have highlighted its relevance (doi: 10.1111/1759-7714.13978). Patients with elevated spontaneous IFN-γ production, as reported in certain conditions like Long COVID (doi: 10.1126/sciadv.adi9379), warrant investigation to assess whether this immune state correlates with prolonged responses to immunotherapy. Moreover, the presence of inborn errors in IFN-γ-related immunity is not typically screened for in cancer patients, despite its potential impact on ICI efficacy (doi: 10.1126/science.adl2016). Our recent findings (doi: 10.1186/s12967-024-06023-8) reveal that suboptimal IFN-γ concentrations may paradoxically promote tumor plasticity and aggressiveness in NSCLC. Low-dose IFN-γ was found to accelerate tumor growth and induce dedifferentiation, contributing to a more heterogeneous and therapy-resistant phenotype. Supporting this, single-cell transcriptomic analyses indicate increased plasticity and immune evasion features in NSCLC cells exposed to low-IFN-γ environments. Similar effects were reported in melanoma models under ICI therapy (doi: 10.1186/s12943-025-02294-x). These data highlight two critical elements for consideration before initiating ICI therapy:(1) the functional capacity of reactivated T cells to produce sufficient IFN-γ, and (2) the tumor's responsiveness to IFN-γ signaling. Notably, impaired ICI efficacy might be partially mitigated by exogenous IFN-γ administration. A Phase 1 study combining IFN-γ with nivolumab (anti-PD-1) in solid tumors confirmed the safety of the approach, though further studies are needed to evaluate its efficacy (doi: 10.1038/s41467-023-40028-z).
Recent work by Mazzaschi et al. demonstrated that longitudinal integration of blood-based immune markers with radiomic descriptors can identify patterns of acquired resistance to ICI therapy (doi: 10.1158/1078-0432.CCR-24-1926). In the same direction, we conducted a comprehensive immune profiling study combining phenotypic and functional analysis of peripheral blood immune components to identify correlates of sustained ICI response. Upon ex vivo stimulation with CD3/CD28/CD2 activators, T cells from Fast Progressor patients displayed a delayed IFN-γ response compared to those from Long Responder patients, suggesting an impaired ability to mount rapid cytokine responses. In conclusion, PD-L1 expression is an insufficient predictor of long-term benefit from ICIs and does not account for T cell reactivation potential. We propose that measuring IFN-γ production following ex vivo peripheral T cell stimulation could serve as a functional biomarker to stratify patients and personalize immunotherapy based on individual immune responsiveness. The results obtained may lay the groundwork for novel diagnostic tools and larger clinical studies.

Aims
The primary objective of this project is to evaluate baseline IFN-γ production by peripheral T cells as a functional biomarker to predict response to immune checkpoint inhibitors (ICIs) in patients with advanced NSCLC. A secondary aim of the project is to investigate the direct effect of anti-PD-1/PD-L1 antibodies on T cell function and to define experimental conditions capable of enhancing IFN-γ production in T cells from patients with delayed or suboptimal responses.

Experimental plan
Aim 1.

PBMCs will be collected from patients at baseline, prior to the initiation of ICI therapy. These cells will be stimulated using a panel of non-specific activators, including IL-2, CD3/CD28/CD2 beads, phytohemagglutinin (PHA), and others (doi: 10.1016/j.cels.2024.11.007). In parallel, antigen-specific stimulation will be performed using NSCLC tumor cell lysates. IFN-γ production will be quantified at different time points (e.g., day 3 and day 7 post stimulation) via flow cytometry. In addition, patients will be screened for inborn errors of IFN-γ immunity that may impact immune responsiveness.

Aim 2.
Baseline PBMCs—both stimulated and unstimulated—will be exposed to ICIs in vitro, and IFN-γ production will be assessed as described above. These data will be compared to IFN-γ production from PBMCs collected from the same patients after three treatment cycles. Longitudinal sampling will also include PBMCs collected at the time of disease progression and, for responding patients, after one year of treatment. Comprehensive PBMC phenotyping, tumor burden evaluation, and IFN-γ production kinetics will be integrated to develop "digital twins" of the peripheral immune system. These computational models will be used to simulate and optimize personalized immunotherapeutic schedules, including T cell stimulation protocols and ICI regimens.

Techniques. Clinical data collection and database creation. PBMC isolation. 2D cell cultures of PBMC and T-cell stimulation. Molecular analysis: flow cytometry, sorting, ELISA test, ELISPOT, whole exome sequencing and analysis, in silico simulation.