

## Immune profiling of cancer patients in the Spanish Immunotherapy Registry of Cardiovascular Toxicity (SIR-CVT)

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**Background:** Immune checkpoint inhibitors (ICIs) have revolutionized cancer treatment by enhancing the immune response against tumor cells. However, their mechanism of action can lead to immune-related adverse events (irAEs), including myocarditis, a rare but life-threatening complication. The role of CD69+ regulatory T cells (Treg) in maintaining immune tolerance has been highlighted in cardiovascular diseases. This study investigates the immune profile of cancer patients undergoing immunotherapy, aiming to identify biomarkers associated with cardiovascular toxicity.

**Purpose:** To analyze immune cell dynamics in cancer patients receiving ICIs and evaluate the potential of CD69 expression as a biomarker for cardiovascular risk.

**Methods:** This sub-study is part of the SIR-CVT study. Peripheral blood samples were collected from 215 cancer patients before ICI-treatment initiation and at different time points (2-4 weeks, 10-12 weeks, 6 months, and 1 year). Flow cytometry was performed to characterize lymphocyte subsets, with a focus on Treg (CD4+FOXP3+CD69+) and proinflammatory Th17 cells (CD4+IL-17+). Patients were stratified into high- and low-cardiovascular risk groups based on CD69 expression (1, 2). Differences were compared by ANOVA. Bioinformatic analysis of SIR-CVT patients stratified by CD69 expression according to the data GeoMean and smooth conditional means are shown (using Loess method).

**Results:** The median age was 66 ±12 years (68% male, 83% had at least one cardiovascular risk factor and 32% had previous cardiac disease). The most common malignancies were none-small cell lung cancer (57%), breast cancer (7%) and melanoma (7%). ICIs included antiPD-1 (67%), antiPD-L1 (24%) and antiCTLA-4 (9%). While total CD4+ T cells did not show significant changes (Figure 1A), patients exhibited a significant decline in activated Treg (CD4+FOXP3+CD69+) within the first 2-4 weeks of treatment (Fig. 1B), suggesting a systemic loss of immune tolerance. Patients with lower baseline CD69 expression experienced a pronounced decline, suggesting increased susceptibility to cardiovascular toxicity (Figure 1C). As a consequence, cytotoxic CD8+ T cells showed an increase after 3 weeks, which became significant at weeks 11 and 26 after ICIs (Fig. 2A). Additionally, a steady increase in proinflammatory Th17 cells was observed over time, becoming significant at one year (Fig. 2B). Bioinformatic analysis confirmed distinct immune profiles between high- and low-risk patients (Fig. 2C).

**Conclusion:** This study reveals dynamic immune shifts during immunotherapy, characterized by a decline in protective Treg and an increase in Th17 cells, which may be responsible of immune-related cardiovascular toxicity. Stratification of patients by CD69 expression could help as a predictive tool for identifying those at higher risk of developing irAEs. These findings pave the way for personalized strategies to stratify cardiovascular toxicity risk in patients undergoing immunotherapy

Figure 1

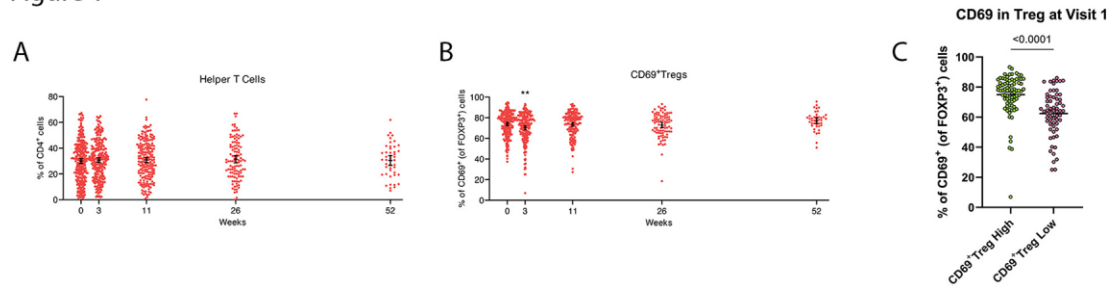


Figure 2

