

Immune Profile Of Cancer Patients In The Spanish Immunotherapy Registry Of Cardiovascular Toxicity (SIR-CVT) During The Course Of Treatment

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Introduction/Purpose

The advancement of immunotherapies has shifted the standard of care in cancer treatment. Immune checkpoint inhibitors (ICIs), such as monoclonal antibodies targeting PD-1, PD-L1 and CTLA-4, stimulate the patient's immune system, through inhibition of regulatory T cells, to attack cancer cells that evade the immune response. However, the mechanism of action of ICIs can also lead to undesirable events (irAEs). During the mechanism of action of ICIs, autoimmune T cell clones are activated. As a consequence, many systemic autoimmune diseases may arise, including myocarditis, which is one of the rarest but most fatal irAEs with a mortality rate of up to 50%. The protein CD69, known as early leukocyte activation antigen, is a type II C-lectin membrane receptor able to inhibit the differentiation of Th17 cells. It is expressed by all hematopoietic cells but erythrocytes upon activation. A subset of Treg (CD4+CD25+) cells with constitutive expression of CD69 has been characterized, which have immuno-regulatory functions and a key role in the maintenance of peripheral tolerance. The low expression of CD69 in this population has been correlated with a worse outcome in those patients who have suffered from different cardiovascular diseases, such as atherosclerosis, acute myocardial infarction and autoimmune myocarditis.

This sub-study, part of the Spanish Immunotherapy Registry of Cardiovascular Toxicity (SIR-CVT), aims to investigate the immune profile of cancer patients' peripheral blood before and during the course of treatment with immunotherapies. The registry focuses on the potential cardiovascular toxicities associated with these therapies, and aims to provide insight into the underlying mechanisms of these toxicities.

Methods

Patients were recruited from various hospitals and blood samples were collected before and after 2-4

weeks, 10-12 weeks, 6 months, and 1 year of starting immunotherapy. Flow cytometry analysis was performed on the blood samples from 103 patients receiving immunotherapy. The data was acquired in a LSRFortessa (BD) instrument and analyzed using FlowJo10.1 software.

Results

Patients show a significant increase in total CD4 T-cell population 6 month and a year after the immunotherapy treatment started (Figure 1 A). However, in the first weeks of treatment, a systemic activation of the immune system is observed due to a very significant drop in activated regulatory T cells (CD4+Foxp3+ CD69+) (Figure 1 B). Moreover, there is a slow but steady increase in the cytokine-secreting T cells which becomes significant one year after the initiation of the treatment (Figure 1 C).

Based on preliminary data, stratification of the patients results in two different groups according to the CD69 expression of their regulatory T lymphocytes at baseline (before starting treatment): high risk (low CD69 expression) or low risk (high CD69 expression), regarding the susceptibility to the development of cardiotoxicity due to immunotherapy treatment (Figure 2 A). Bioinformatic analysis showed significant differences between these two groups of patients – a lower representation of the Treg population could be observed those patients belonging to the high-risk group when compared to low-risk patients in baseline (Figure 2 B). Moreover, throughout the course of treatment, these high-risk patients have lower numbers of naïve helper lymphocytes and, on the contrary, higher numbers of memory helper lymphocytes (Figure 2 B).

Interestingly, we have observed those patients within the high-risk group suffer a more drastic drop in CD69 expression at the first visit (2-4 weeks) since treatment started, whereas low-risk patients present a more constant CD69 expression throughout treatment (Figure 2 B). This phenomenon of loss of peripheral tolerance usually overlaps temporally with the development of irAEs. If we study the kinetics of populations over time, in high-risk patients we observe an increase in the proinflammatory Th17 cell population (Figure 2 B), as well as those cells double positive for IL-17 and IL-22, which have been described to be closely related to autoimmunity phenomena.

Conclusion(s)

This study presents a new understanding of the dynamics in peripheral blood lymphocytes during cancer immunotherapy. Results suggest a decrease in activated regulatory T (CD4+FOXP3+CD69+) cells, which play a key role in maintaining immune homeostasis, and an increase in effector T cells, as autoimmune Th17 subset. These shifts can cause disruption in immune homeostasis and may result in autoimmunity phenomena, including myocarditis. By stratifying patients in two different groups regarding susceptibility to develop irAEs, preliminar data suggest that CD69 low-expressing patients experiment a high downregulation of this protein and, therefore higher susceptibility to develop cardiotoxicities. Data also show that immunotherapy treatment could lead to chronic inflammation in susceptible patients. Combining these findings with clinical data, we have the potential to identify biomarkers that could predict or prevent adverse events and ultimately improve patient outcomes.

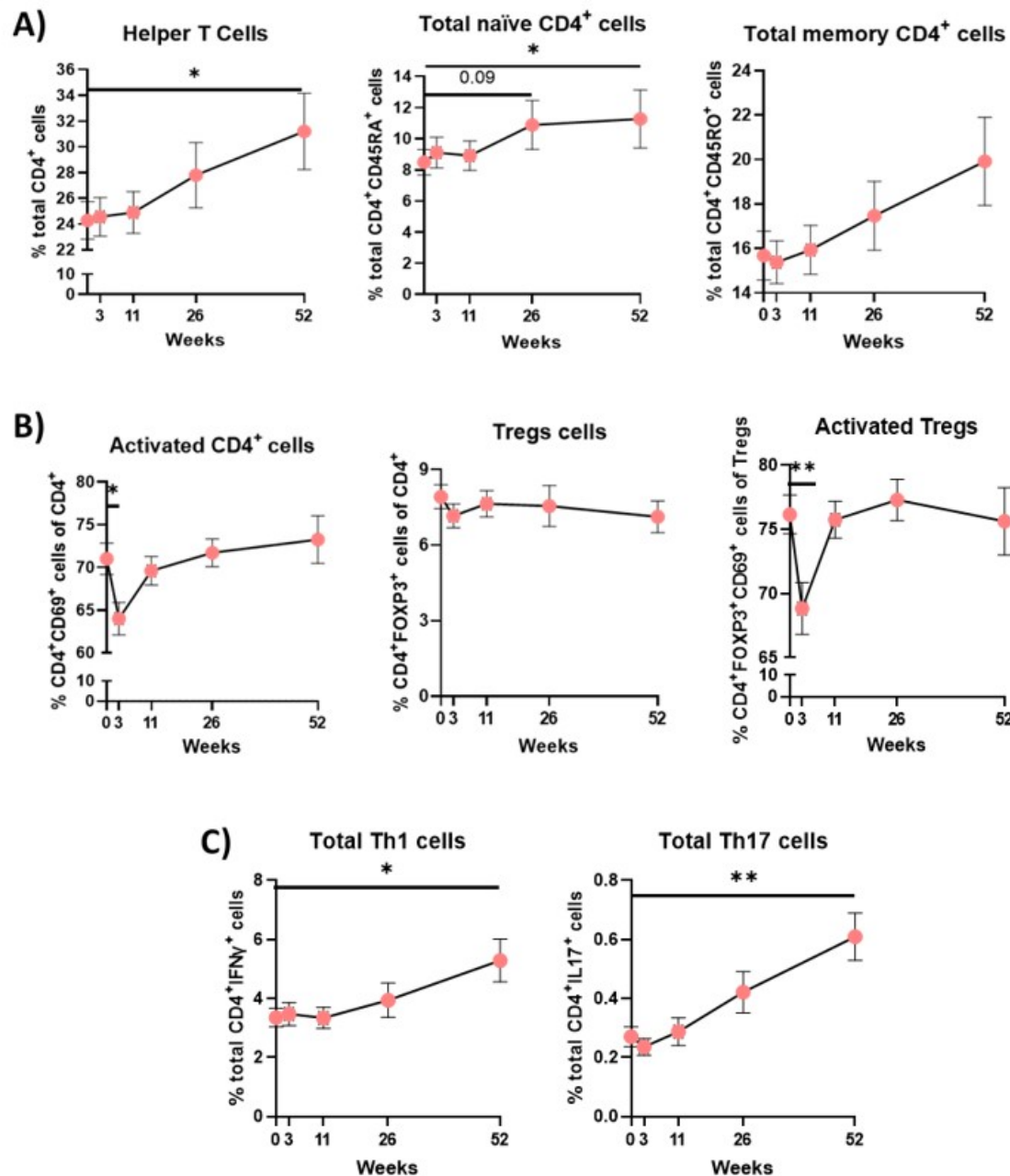


Figure 1. Immune profile of blood samples from patients treated with ICI therapy. Percentages of peripheral blood lymphocytes analyzed by flow cytometry in longitudinal assays from patient's blood samples with cancer before ICI treatment (n=103), after 2-4 weeks (n=86), 10-12 weeks (n=76), 6 months (n=41), and 1 year (n=18) since the first therapy cycle. A) Percentages of total CD4⁺, CD4⁺CD45RA⁺ and CD4⁺CD45RO⁺ cells of PBLs. B) Percentages of CD4⁺CD69⁺ and CD4⁺FoxP3⁺ cells CD4⁺-gated, and CD4⁺FoxP3⁺CD69⁺ cells gated in CD4⁺FoxP3⁺ cells. C) Percentages of CD4⁺IL17⁺ cells and CD4⁺IFN γ ⁺ cells gated in CD4⁺ cells. Data is represented as mean $\pm$ SEM. Statistical differences between means was measured using mixed-effects analysis of variance followed by Dunnett's post-hoc test. * P < 0.05, ** P < 0.01, ***p < 0.001.

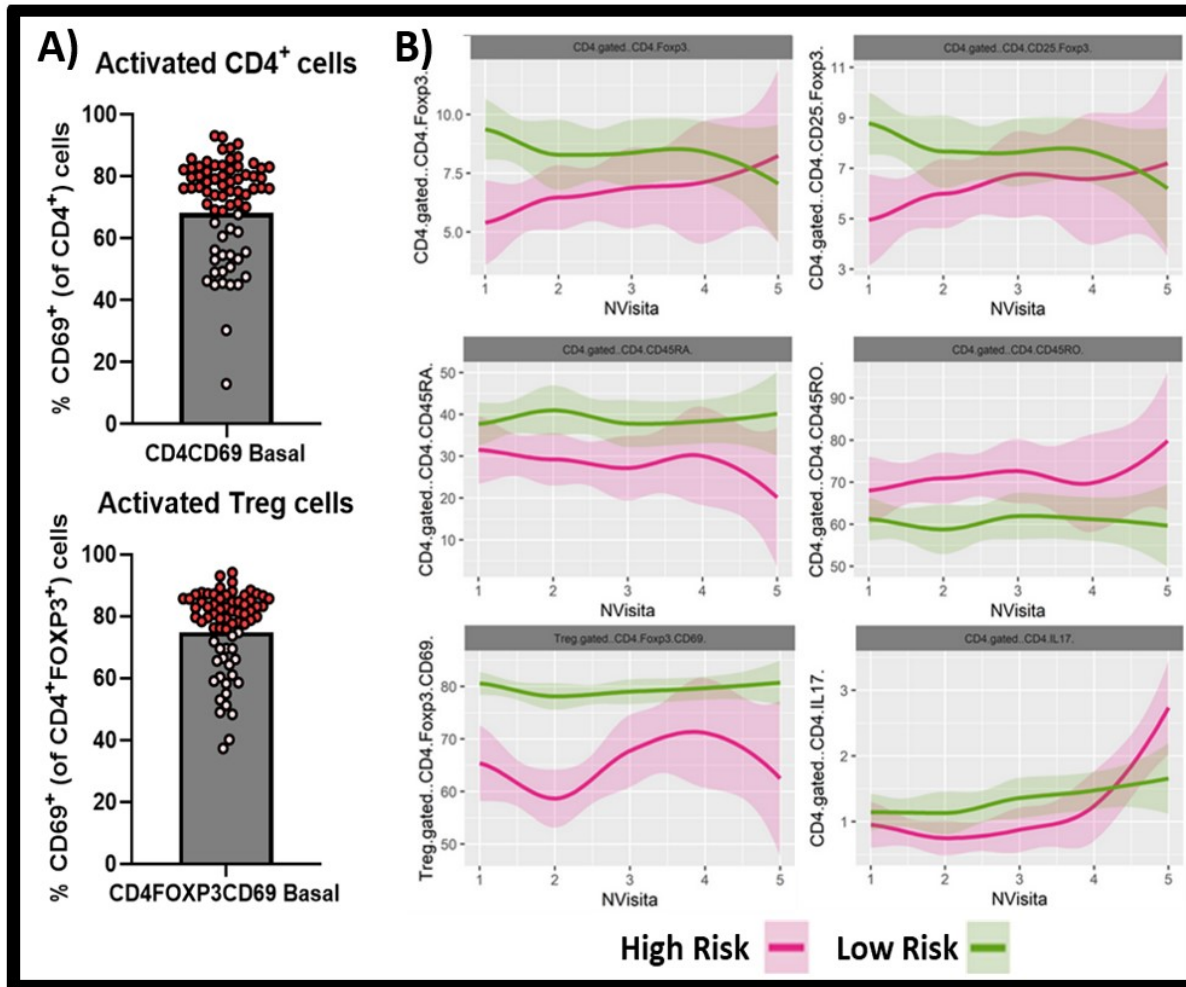


Figure 2. Bioinformatic analysis of SIR-CVT patients stratified by CD69 expression. Patients were divided in two different groups regarding CD69 expression: high risk (low CD69 expression) or low risk (high CD69 expression) A) Percentages in baseline (before immunotherapy treatment started) of CD4+CD69+ gated in CD4+ cells, and CD4+FoxP3+CD69+ cells gated in CD4+FoxP3+ cells. B) Smooth conditional means (using method loess) of parameters in time of CD4+FoxP3+ cells, CD4+CD25+FoxP3+ cells, CD4+CD45RA+ cells, CD4+CD45RO+ cells and CD4+IL17+ cells all CD4+-gated, and CD4+FoxP3+CD69+ cells gated in CD4+FoxP3+ cells. The X axis represents basal in 1, visit 1 (2-4 weeks) in 2, visit 2 (10-12 weeks) in 3, visit 4 (6 months) in 4 and visit 5 (one year) in 5, and high-risk patients are represented in red and low-risk patients in green.