Pre-existing immunity as predictive biomarker in cancer immunotherapy for NSCLC patients

Anastasia Xagara[1], Maria Goulielmaki[2],Sotirios Fortis[2], Fillipos Koinis[3], Evangelia Chantzara[3],Alexandros Kokkalis[3], Christodoulopoulos G[3], Giannis Samaras[3], Vasilios Papadopoulos[3], Vasilios Georgoulias[4], Constantin Baxevanis[2], Athanasios Kotsakis[1,3]

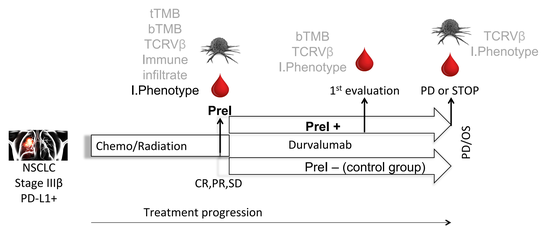
1. Medical Oncology Department, Laboratory of Oncology, University of Thessaly, Larissa, Greece, 2.Cancer Immunology and Immunotherapy, Agios Savvas - Anticancer Hospital, Athens, Greece, 3. Medical Oncology Department, University Hospital of Larissa, Larissa, Greece, 4. Emeritus Professor, University General Hospital of Heraklion, Heraklion, Greece

Disclosure statement: I have no conflicts of interest to declare

Backround: Pre-existing immunity that describes the endogenous tumor-specific adaptive immunity, before treatment may represent a valuable novel predictive biomarker for ICI treatment [1,2,3]. In this study we estimate the potential value of pre-existing cancer-antigen specific T- cells as circulating predictive biomarkers. Additionally, we evaluate the major differences of known immune cell phenotypes between Pre-existing positive (PreI+) and Pre-existing negative (PreI-) NSCLC patients in circulation.

Methods

Blood was collected before initiation of immunotherapy from 25 NSCLC patients stage IIIb, PD-L1+ that receiving Durvalumab. PBMCs were isolated with Ficoll density gradient centrifugation including 15 healthy donors (HD). PreI was calculated by detecting endogenous IFNg expressing cells after in- vitro co-cultures of PBMCs with hTERT, MAGEA1, NY-ESO-1 and Survivin antigens. Immunophenotyping was performed by multi-color flow cytometry using antibodies against CD3, CD4, CD8, CD45RA, CD45RO,CCR7,PD-1,PD-L1 for CD4 and CD8 T-cells and CD3, CD4, FoxP3,CD25,CD127,CTLA-4,CD39 for Tregs.



Results

1. 40% of NSCLC patients secrete IFN-g indicating TAA PreI+ T-cells

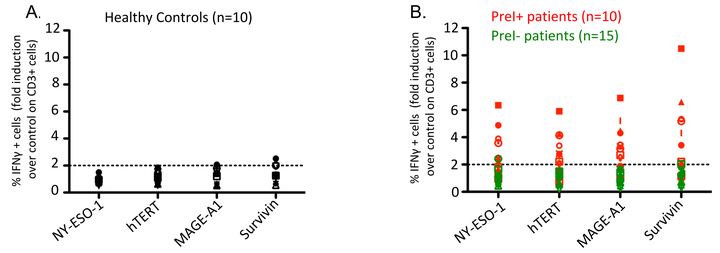


Figure 1. Percentages of $CD3^+IFN\gamma$ secreting cells for (A) healthy donors and (B) NSCLC patients, for all 4 TAA tested as fold induction over control. Black dashed line represents the cut off that was set considering $CD3^+IFN\gamma$ secreting cells for healthy donors, In red 10 $PreI^+$ NSCLC patients, in green 15 $PreI^-$ NSCLC patients. Each data point respesent the SEM of three indepented replications.

2. Detection of TAA specific T-cells is correlated positively with response to ICI

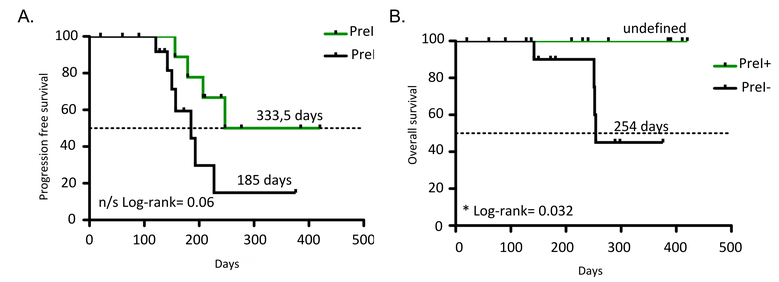


Figure 2. Kaplan Maier survival analysis of PreI- (black line) and PreI+ (green line) NSCLC patients treated with Durvalumab (A) PFS analysis (Log-rank = 0.06, median 185 and 333,5 days) (B) OS analysis (*Log-rank = 0.032, median 254 and undefined days).

3. Patients with PreI+ and low levels of CTLA-4 Tregs have a survival benefit

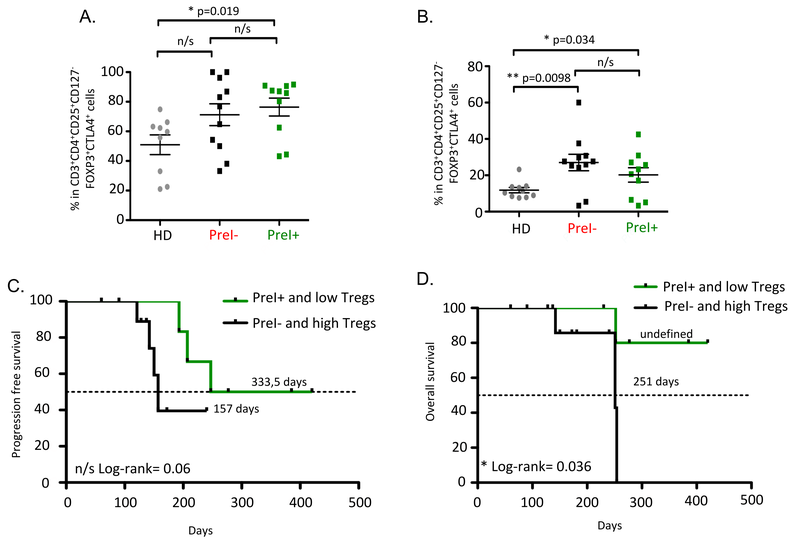


Figure 3. Percentages of (A) CTLA-4+ Tregs and (B) CD39+ Tregs in helthy donors, PreI- and PreI+ patients. Kaplan Meier survival curves for (C) PFS (n/s Log-rank 0,102, n=8 vs n=9, median 333,5 and 157 days) and (D) OS (*Log-rank 0,036, n=8 vs n=9, median undefined and 251 days) in NSCLC patients with PreI+ and low CTLA-4 Tregs (in green) vs PreI- patients and high CTLA-4 Tregs (in black) .

Funding : This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH-CREATE-INNOVATE (project code :T2ΕΔΚ-02218)

4. Patients with PreI+ have significant higher levels of $CD8^+$ T-exhausted cells

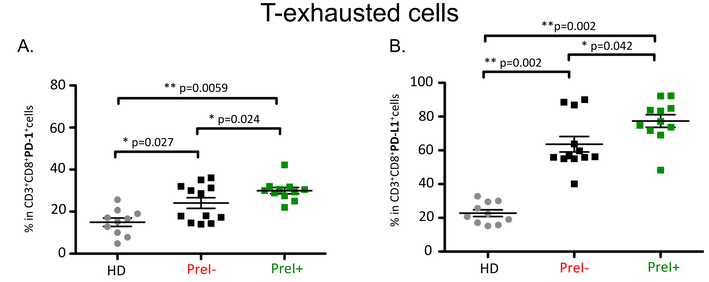


Figure 4. Percentages of (A) $CD3^+CD8^+PD\text{-}1^+$ and (B) $CD3^+CD8^+PD\text{-}L1^+$ CD39+ T-cells in circulation of healthy donors and PreI- and PreI+ patients before ICI treatment.

5. $CD8^+$ T-effector cells are detected in higher frequency in $PreI^+$ patients comparing to $Prei^-$

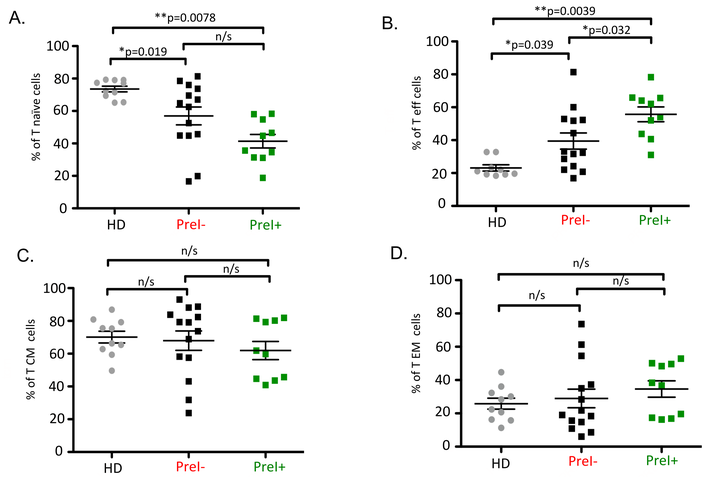


Figure 5. Percentages of (A) $CD3^+CD8^+CD45RA^+CD45RO^-CCR7^+$T-naïve cells (B) $CD3^+CD8^+CD45RA^+CD45RO^-CCR7^-$ T-effector (C) $CD3^+CD8^+CD45RA^-CD45RO^+CCR7^+$T central memory and (D) $CD3^+CD8^+CD45RA^-CD45RO^+CCR7^-$T effector memory cells in healthy donors, PreI – and PreI + patients before treatment.

Conclusions : Pre-existing tumor antigen specific T-cells in circulation before initiation of immune checkpoint inhibitors in NSCLC patients serve as a good prognostic factor of response. Further analysis in immune phenotypes indicates major differences favoring to a more responsive immune status of $PreI^+$ patients. Future analysis on their kinetics during ICI therapy may reveal a stronger prediction algorithm of response.

References: 1. *Gridelli, C. et al, Br J Cancer* 122, 1461–1466 (2020)
2. *Kotsakis A. et al, Ann Oncology* 23,442-449 (2012)
3. *Mavroudis D. et al, Oncology* 70, 306–314(2006)

Contact information : Xagara Anastasia, e-mail: **xagaraa@hotmail.com**