Peripheral pre-existing T-cell immunity as predictive biomarker in cancer immunotherapy for NSCLC patients

Anastasia Zagaras1, Sotiros Fortis2, Maria Gouliemaliki3, Fillipos Koinis2, Evangelia Chantzaras1, Giannis Samaras2, Vasilios Papadopoulos2, Vasilios K. Sorgouros2, Konstantin Baxevanis2, Athanasios Kotsakis4

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. Ermoupolis Private Hospital General Hospital of Heraklion, Heraklion, Greece

Disclosure statement: I have no conflicts of interest to declare.

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

Methods

Blood was collected before initiation of immunotherapy from 25 NSCLC patients stage IIIb, PDL1+ that receiving Durvalumab. PBMCs were isolated with Ficol density gradient centrifugation including 15 healthy donors (HD). Pre+ was calculated by detecting endogenous IFNγ expression in vitro, co-culturing PBMCs with tTMB, MAGEA1, NY-ESO-1 and Survivin antigens. Immuno-phenotyping was performed by multi-color flow cytometry using antibodies against CD4, CD8, CD45RA, CD45RO, CCR7, PD-1, PD-L1 for CD4 and CD8 T-cells and CD3, CD4, FoxP3,CD25,CD127,CTLA-4,CD93 for Tregs.

Results

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

Figure 1. Percentages of CD3+IFNγ secreting cells for (A) healthy donors and (B) NSCLC patients, for all a TAA (Immuno-phenotyping) in vitro and control. Black dashed line represents the cut off that was set considering CD3+IFNγ secreting cells for healthy donors, in red 10 Pre+ NSCLC patients, in green 15 Pre− NSCLC patients. Each data point represents the SEM of three independent replications.

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

Figure 2. Kaplan Meier survival analysis of Pre+ (black line) and Pre+ (green line) NSCLC patients treated with Durvalumab (A) PFS analysis (Log-rank = 0.05, median 185 and 333.5 days) (B) OS analysis (*Log-rank = 0.03, median 254 and undefined days).

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

Figure 3. Percentages of (A) CTLA-4+ Tregs and (B) CD93+ Tregs in healthy donors, Pre+ and Pre− patients. Kaplan Meier survival curves for (C) PFS (n≥ Log-rank 0.102, n≤ vs n≥, median 333.5 and 157 days) and (D) OS (*Log-rank 0.036, n≤ vs n≥, median undefined vs 251 days) in NSCLC patients with Pre+ and low CTLA-4 Tregs (in green) vs Pre− patients and high CTLA-4 Tregs (in black).

4. Patients with Pre+ have significant higher levels of CD8+ T-exhausted cells

Figure 4. Percentages of (A) CD3-CD8-PD-1- and (B) CD3-CD8-PD-1-L-1- CD3+ T-cells in circulation of healthy donors and Pre+ and Pre− patients before ICI treatment.

5. CD8+ T-effector cells are detected in higher frequency in Pre+ patients comparing to Pre−

Figure 5. Percentages of (A) CD3-CD8-CD45RA-CD69-CCR7+ T-naive cells (B) CD3+CD8+CD45RA+CD69+CCR7+ T-effector (C) CD3+CD8+CD45RA+CD69+CCR7+ T-effector memory cells in healthy donors, Pre+ and Pre− 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 Pre+ patients. Future analysis on their kinetics during ICI therapy may reveal a stronger prediction algorithm of response.


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

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: T2EAK-02218)