Multiplex analysis of the immune environment before and after neoadjuvant durvalumab as a prognostic factor in resectable non-small cell lung cancer (NSCLC) in the IFCT-1601 IONESCO phase 2 trial.

D Damotte[1,2], Y Velut[1], B Burroni[1,2], G Birsen[2], J Lavergne[1], A Lupo[1,2], I Cremer[2], J Mazieres[3], J Cadranel[4], G Zalcman[5], R Gervais[6], G Jeannin[7], O Molinier[8], T Egenod[9], O Carre[10], C Berndt[11], A Langlais[12], F Morin[11], V Westeel[13], M Wislez[14]

[1] U1138, Centre de Recherche des Cordeliers 75006 - Paris/FR, [2] Pathology, Hôpital Cochin, 75014 - Paris/FR, [3] Thoracic Oncology Department, Hôpital Larrey, 31059 - Toulouse/FR, [4] Pneumology, Hopital Tenon, 75020 - Paris/FR, [5] Department Of Thoracic Oncology, Hôpital Bichat, 75018 - Paris/FR, [6] Pneumology, Centre Francois Baclesse, 14076 - Caen/FR, [7] Thoracic Oncology, Hôpital Gabriel Montpied, 63003 - Clermont-Ferrand/FR, [8] Pneumology, Centre Hospitalier Du Mans, 72037 - Le Mans/FR, [9] Thoracic Oncology, Hopital Dupuytren, 87042 - Limoges/FR, [10] Pneumology, Clinique de l'Europe Amiens, 80000 - Amiens/FR, [11] Clinical Research Unit, French Cooperative Thoracic Intergroup, 75009 - Paris/FR, [12] Biostatistics, French Cooperative Thoracic Intergroup, 75009 - Paris/FR, [13] Pneumology, Hopital Jean Minjoz, 25030 - Besançon/FR, [14] Pneumo-oncology, Hôpital Cochin, 75014 - Paris/FR

Background

Immune checkpoint inhibitors (ICI) are currently included in the peri-operative standard of care for NSCLC with the objective of a curative strategy. In early stages of NSCLC, biomarkers predicting ICI efficacy should be more stringent than PD-L1 tumoral expression in order to improve the benefit-toxicity ratio. We deeply analyzed the tumor microenvironment of patients included in the IONESCO multicenter phase 2 trial (stage IB>4cm–IIIA, non N2 resectable NSCLC). Diagnostic biopsies and surgical resection specimen after 3 cycles of Durvalumab were available. We previously showed that the % of residual viable tumor cells (RVT) was associated with disease free survival (DFS) and overall survival (OS). PD-L1 tumor positive score was not correlated to RVT nor survival.

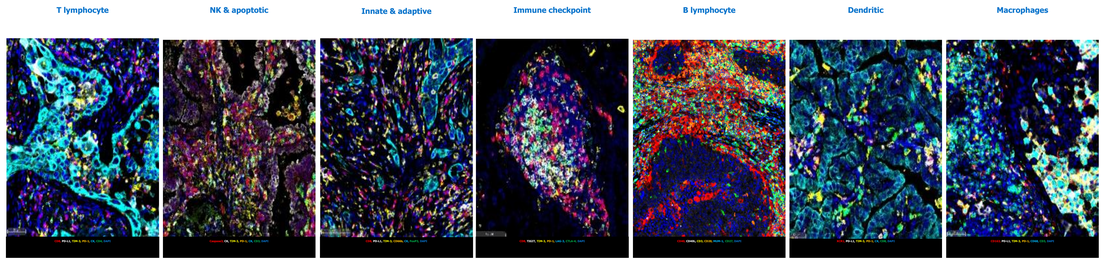
Methods

46 patients were included. Among them, diagnostic biopsy (n=32), surgical resection specimen post durvalumab (n=39) and paired tumor samples (n=31) were analyzed. Immune environment was assessed using 7 quantitative 7-plex immunofluorescence panels generating 349 different cellular phenotypes in 3 different compartments (whole tumor, intra cytokeratin and stroma), focusing on T and B lymphocytes, macrophages, immune checkpoint, NK cells, apoptosis, innate and adaptive immunity, dendritic cells. Densities of cells were quantified using Fluorescent Multiplex immunohistochemistry performed on Leica Bond RX, using OpalTM technology. A fisher's exact test or chi2 test was used for demographics variables and RVT. HRs and 95% CIs were estimated using a Cox model in patients and we excluded early post operative death from this specific *in situ* analysis of the immune environment.

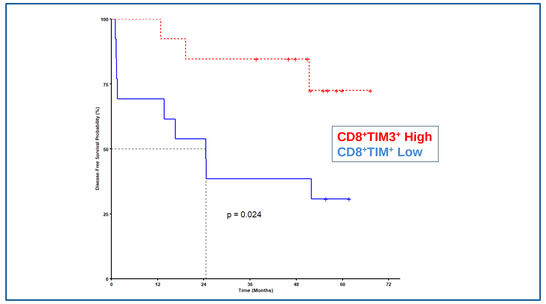
Results

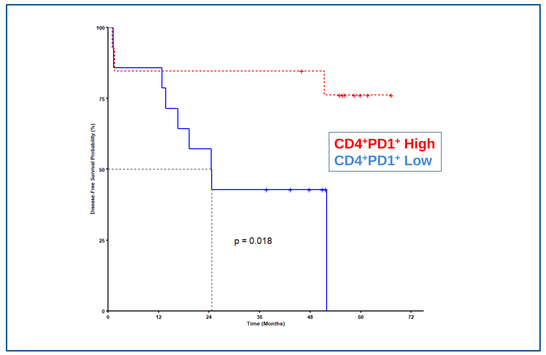
Forty-six patients were eligible (median age 60.9 years); 67% were male, 98% were smokers, and 41% had squamous cell carcinoma. Regarding tumor response, 9% had partial response, 78% had stable disease, and 13% had progressive disease. Among the operated patients (n=43), 41 achieved complete resection (89%, 95% CI 80.1% to 98.1%), 8 achieved MPR (19%) and 4 died within 90 days after surgery. With median follow-up of 4.5 years, 16/31 patients had a disease recurrence. Biopsies before neoadjuvant durvalumab were the most reliable material to predict DFS according to clinical and histological characteristics and to immune populations densities. Histology, sex, stage and survival were significantly associated with intra-tumor densities of CD3, CD8, PD1, TIM3, and CD163 cells in the diagnostic biopsy. DFS was significantly associated with high density of $CD8^+TIM3^+$ (HR=0.44 [0.13-1.45], p=0.024), $CD4^+PD1^+$ (HR = 1.10 [0.32-3.8] (p=0.018), $FoxP3^+$ (HR = 0.22 [0.06-0.82], (0.014) in biopsies and with the high density of $MUM1^+CD27^-$cells in surgical specimen (HR=0.15 [0.03-0.71], p=0.0061). DFS was also significantly associated with $CK^+Caspase3^+$ cells in biopsies. No biomarker was associated with OS even if we found a tendency with $CD4^+$ and $CD8^+$ densities (p=0.056 and p=0.068 respectively).

7-plex and 7 panels representative images

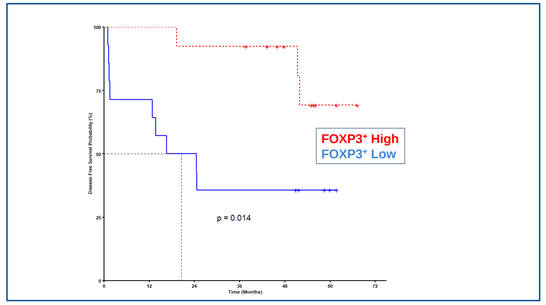


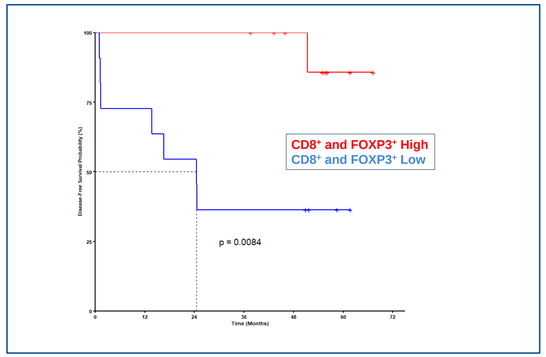
Density of $CD4^+PD1^+$ and of $CD8^+TIM3^+$ in biopsies predict DFS



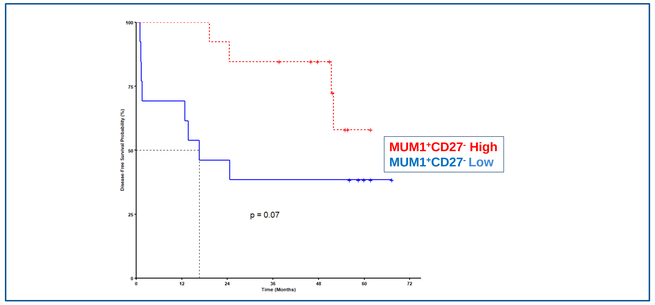


Density of $FoxP3^+$ in biopsies predict DFS

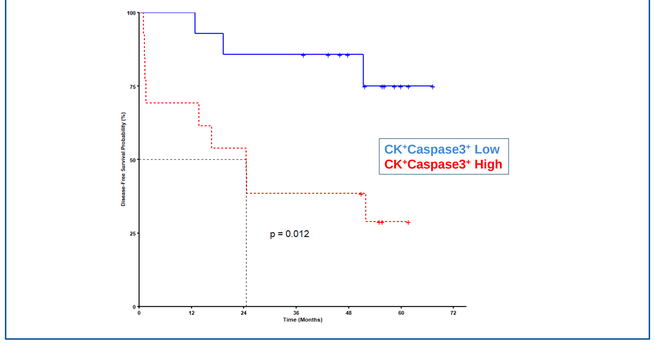




Density of $MUM1^+CD27^-$ Post-Durvalumab therapy predict DFS



Density of tumoral cells Cytokeratin$^+$ expressing Caspase 3 ($CK^+Casp\ 3^+$) in biopsies predict DFS



Conclusion

Multiplex analysis of the immune NSCLC environment of patients treated with neoadjuvant anti-PDL1 allows identification of markers associated with clinical and pathological parameters and with DFS. Our findings highlight the substantial impact of high CD8+TIM3+, CD4+PD1+, and FoxP3+ cell density pre-durvalumab, as well as high MUM1+CD27+ cell density post-durvalumab, on DFS. Interestingly, most markers were found in the pre-therapeutic biopsies and deserve to be assessed in patients treated with neoadjuvant combined immunotherapy and chemotherapy in prospective trials.

*Corresponding author : diane.damotte@aphp.fr
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