DEVELOPMENT OF A MULTIPLEX TEST FOR PREDICTING RESPONSE TO COMBINED IMMUNOTHERAPIES IN PATIENTS WITH METASTATIC MELANOMA

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Background In recent years, advanced melanoma treatment has improved dramatically thanks to the advent of immunotherapy. Particularly immune checkpoint inhibitors (ICI), in some patients, have demonstrated to improve long-term outcomes associated with limited toxicity. However, only a small population of patients achieve a durable response to therapy, owing to the lack of clinically validated predictive biomarkers (reviewed in1). The availability of improved predictive biomarkers may allow the identification of patients who will most benefit from ICI treatment and those who may be susceptible to immune-related adverse events. This difficulty in obtaining clinically relevant predictive biomarkers underscores the complexity of the immune system and the heterogeneity of the tumor microenvironment. In the present study, we take the first steps towards stratification of advanced melanoma patients who received a combination of ICI.

Methods In this retrospective study, from the biobank of the Istituto Nazionale Tumori IRCCS Fondazione G. Pascale, we recovered FFPE skin metastasis samples obtained from 10 melanoma patients with AJCC 8th edition stage IV2 subsequently treated with combined ICI, enrolled from September 2016 to November 2017. According to the Response Evaluation Criteria in Solid Tumors (RECIST 1.1),2 4 patients achieved response to the treatment, while 6 patients were non-responders. A single FFPE tumor tissue of each patient was stained by DAPI counterstaining and whole slide fluorescent scanning.

Results Responders patients showed a statistically significant increase in CD8+ single-positive cell frequency compared to non-responders (figure 2A and 2C). Non-responder patients displayed a statistically significant increase of PD-L1+ single-positive cell frequency, as well as statistically significant increased frequency of double CD8+PD-L1+ positive cells, previously found to be a poor prognostic in multiple cancer types,3,5 and triple positive cells (figure 2B and 2C).

Conclusions We show here preliminary evidence of the predictive value of a spatial biomarker signature in patients who underwent combined ICI therapy for advanced melanoma. To further demonstrate clinical relevance, a more detailed analysis using larger retrospective and prospective cohorts is ongoing.

REFERENCES
gene-3 (LAG-3, magenta), programmed death-ligand 1 (PD-L1, green), cluster of differentiation 8 (CD8, red) and DAPI (blue) on FFPE melanoma skin metastasis samples obtained from patients prior to their first cycle of combined immune checkpoint inhibitors (ICI) treatment. Three patients were classified as Responder (R) (A) and three as Non-Responder (NR) (B) based on their response evaluation criteria. Scale bar: 20 μm. Yellow arrows (A-B) indicates examples of double-positive CD8+PD-L1+ cells, prominently present in NR patients (B-C). (C) Quantification of the number of CD8+ (upper left), PD-L1+ (upper right), CD8+PD-L1+ (bottom left) and CD8+LAG-3+PD-L1+ (bottom right) cells from R and NR patients. * p<0.05, *** p<0.001, Unpaired t-test