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Disclosure

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Sex-based differences of the tumor mutational burden and T-cell inflammation of the tumor microenvironment

It has been recently reported the validation of a new biomarker, with strong predictive value for response to pembrolizumab, based on the tumor mutational burden (TMB) and a gene expression signature of 18 genes (T-cell-inflamed GEP) [1].

TMB is an indirect measure of tumor antigenicity generated by somatic tumor mutations [2]. T-cell-inflamed GEP signature includes genes indicative of an ongoing Th1 and cytotoxic CD8+ T-cell-driven immune response, including IFN-γ signaling, cytolytic activity, antigen presentation, and T-cell trafficking, as well as adaptive inhibitory molecules such as programmed cell death protein 1 (PD-1)/programmed death-ligand 1 and indoleamine 2,3-dioxygenase 1, that are co-regulated within tumor microenvironment [3].

Both tumor antigenicity and a T-cell-driven inflammation of the tumor microenvironment are necessary elements to obtain a response to immune-checkpoint inhibitors [4]. Jointly analyzing these two variables, the new biomarker categorizes tumors in four different groups [(i) GEP low and TMB low, (ii) GEP low and TMB high, (iii) GEP high and TMB low, (iv) GEP high and TMB high], characterized by a different degree of responsiveness to pembrolizumab, regardless of tumor histotype. The predictive value of this new biomarker has been validated in three independent cohorts of patients with 22 different tumor histotypes, treated with pembrolizumab.

We previously described that the modality through which women and men with cancer respond to immunotherapies is different, with men obtaining a significantly larger benefit than women from anti-CTLA4 or anti-PD-1 monotherapy compared with chemotherapy, regardless of tumor type [5]. We therefore re-analyzed available patient-level data, used to validate the new biomarker [1], according with patients' sex.

We found that tumors of male and female patients were differently distributed among the four biomarker-defined groups (Figure 1A). In the whole patient population, the percentage of tumors with low levels of either TMB and GEP score—a condition that strongly predicts for absence of response to pembrolizumab—was nearly double in women as compared with men (GEP^{lo} TMB^{lo} 29% in women versus 15% in men, prevalence ratio 1.9, 95% confidence interval 1.22–2.96). By contrast, the percentage of tumors characterized by high TMB and GEP score—that is associated with a high probability of response to pembrolizumab—was almost halved in women compared with men (GEP^{hi} TMB^{hi} 26% in women versus 42% in male, prevalence ratio 0.61, 95% confidence interval 0.43–0.88).

These differences were observed in all the three independent cohorts of patients analyzed to validate the biomarker [1] (i.e. melanoma, head and neck squamous cell carcinoma and the pancancer cohort, that includes 20 different cancer types; P-heterogeneity: 0.47), with the largest difference observed in the cohort of patients with melanoma (Figure 1B). Sex retained a significant association with the biomarker-defined groups after controlling for age and tumor histotype in a logistic multivariable model ($P = 0.032$).

Such large sex-based differences in both TMB and T-cell inflammation of the tumor microenviroment, which are key elements of the anticancer immune-response and are strongly associated with responsiveness to pembrolizumab, further confirm the relevance of sex-dimorphism in spontaneous as well as drug-enhanced anticancer immune responses. Confirmation of the predictive value for response to pembrolizumab of the new
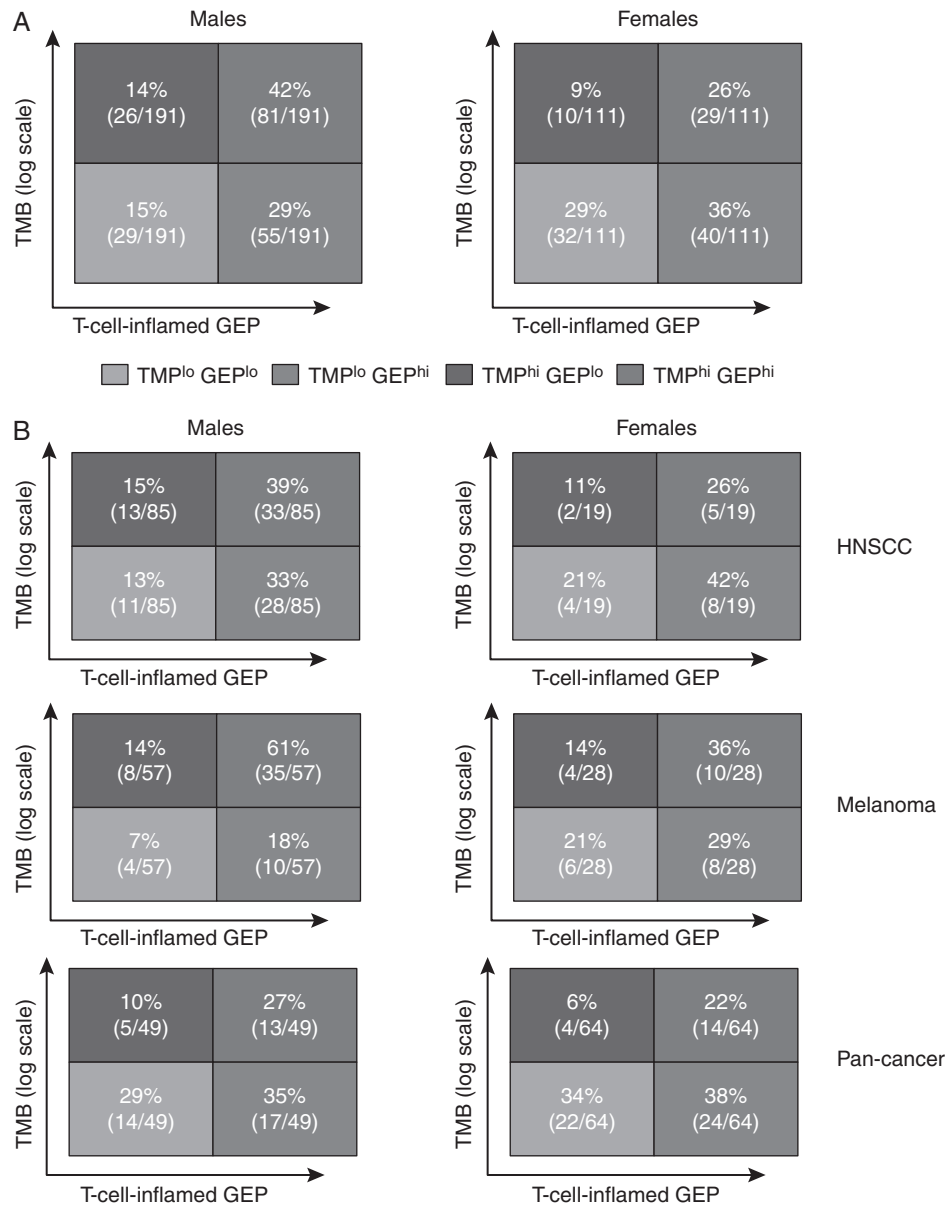
Figure 1. (A) Tumors distribution among the four biomarker-defined groups according to patients' sex. It reports the percentage and the absolute number of tumors for each of the four biomarker-defined groups, according to patients' sex. The four biomarker groups are as follows: GEP low and TMB low (GEP[lo] TMB[lo]), GEP low and TMB high (GEP[lo] TMB[hi]), GEP high and TMB low (GEP[hi] TMB[lo]), GEP high and TMB high (GEP[hi] TMB[hi]). TMB and T-cell-inflamed GEP cutoffs used to define the four groups are the same utilized in the original paper [1]: TMB[hi] and TMB[lo] groups were defined by values greater than or equal to and less than Youden Index-associated cut points (102.5, 86, and 191.5 for pan-cancer, HNSCC, and melanoma cohorts, respectively); GEP[hi] and GEP[lo] groups were defined by cutoffs greater than or equal to and less than −0.318, respectively [1]. (B) Tumors distribution among the four biomarker-defined groups according to patients' sex and tumor cohort. It reports the percentage and the absolute number of tumors for each of the four biomarker-defined groups, according to patients' sex and tumor cohort. The three tumor cohorts are pan-cancer, HNSCC, and melanoma. The pan-cancer cohorts includes 20 different cancer types [1]. TMB and T-cell-inflamed GEP cutoffs used to define the four groups were the same utilized in the original paper [1]. TMB, tumor mutational burden; GEP, gene expression profile; HNSCC, head and neck squamous cell carcinoma.

biomarker separately in male and female patients would be warranted.

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Machine learning-based predictors for immune checkpoint inhibitor therapy of non-small-cell lung cancer

Immunotherapy targeting programmed cell death protein 1/programmed death-ligand 1 (PD-1/PD-L1) is a standard of care in the treatment of stage IV non-small-cell lung cancer (NSCLC). However, only a minority of patients responds to anti-PD-1/PD-L1 monotherapy. Tumor-centric predictive biomarkers applicable to small diagnostic specimens such as PD-L1 expression and tumor mutational burden [1] only allow enrichment of cohorts with higher probability of treatment response. Efficacy of immunotherapy is governed by a complex interplay of tumor-intrinsic properties (genomic and epigenomic), the tumor microenvironment, the systemic state of the immune system, and *de novo* or acquired resistance [2]. Capturing this 'cancer-immune set point' [3] requires insight in both tumor biology and the tumor microenvironment. Recent technological advances have allowed studying hundreds of genes in small, diagnostic biopsies, which are the clinically feasible biosample format in most patients with stage IV cancer. Specific signatures have been validated to carry predictive information across cancer types [4, 5].

We set out to explore and validate the predictive value of a machine-learning approach based on archival, formalin-fixed paraffin-embedded tumor biopsies. Patients with advanced or metastatic NSCLC and available surplus routine biopsy specimens were sequentially enrolled to a training ($n = 55$) and validation cohort ($n = 36$; supplementary Table S1, available at *Annals of Oncology* online). All patients had received anti-PD-1 antibodies in second- or further line. Expression analysis of 770 immune-related genes was performed on the NanoString nCounter platform (NanoString Technologies, Inc., Seattle, USA). Clinical end points were best response, time-to-treatment-failure and overall survival following immunotherapy. From the expression data, predictive feature sets were selected by ensemble-based penalized regression techniques and by utilizing previously published expression signatures of immune cell subtypes. Best performing machine-learning techniques and associated hyperparameters were selected by cross-validation from a set of state-of-the-art algorithms.

The feature selection process allowed identifying a subset of approximately 20 of 770 genes that associated with clinical outcome (Figure 1A). We utilized the training cohort to derive prediction models based on the end point of best response following immunotherapy. In the validation cohort, these models successfully identified all 'top responders'. Concordant prediction of clinical benefit by our models identified a subgroup of patients that benefits from immunotherapy ($P = 0.035$, hazard ratio = 0.32, Figure 1B). PD-L1 immunohistochemistry appeared to confer an orthogonal layer of information: Incorporating PD-L1 tumor proportion score (PD-L1 TPS) provided a combined prediction with an even stronger predictive value (favorable/intermediate/unfavorable, $P = 0.006$, Figure 1C). Among patients with PD-L1 positive tumors 10 of 13 were correctly classified (77%); in particular, all patients in this group who did not benefit were correctly identified (3/3 patients, Figure 1D).

Our findings show that machine-learning techniques based on nCounter RNA expression data can be applied to achieve immunotherapy response prediction. This approach appeared to provide information in addition to PD-L1 expression. Limitations of our study include the limited sample size of both training and validation cohorts and missing comparison with the predictive information of tumor mutational burden. The employed platform allows analysis of RNA extracted even from small formalin-fixed paraffin-embedded biopsies. Integration into a standard diagnostic workflow relying on small biopsy specimens is feasible. Thus, nCounter analysis can be cost-effective and integrated into the standard molecular pathology workup to enable rapid clinical decision making in precision immunotherapy of NSCLC.

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