Editorial

Tumor mutational burden as predictive factor of response to immunotherapy

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Comment on: Hellmann MD, Nathanson T, Rizvi H, *et al.* Genomic Features of Response to Combination Immunotherapy in Patients with Advanced Non-Small-Cell Lung Cancer. Cancer Cell 2018;33:843-52.e4.

Submitted Sep 24, 2018. Accepted for publication Sep 30, 2018.
doi: 10.21037/tlcr.2018.10.03
View this article at: http://dx.doi.org/10.21037/tlcr.2018.10.03

In May 2018, Hellmann *et al.* published in *Cancer Cell* journal a retrospective study analyzing the predictive value of tumor mutational burden (TMB) in 75 non-small cell lung cancer (NSCLC) patients treated with the combination of the anti PD-1 inhibitor nivolumab and the anti CTLA-4 antibody ipilimumab in the Check-Mate 012 study. Results confirmed that tumors with high TMB were more prone to have benefit after treatment with nivolumab plus ipilimumab, than those with low TMB. Response rate, durable clinical benefit (DCB) and progression free survival (PFS) were better in patients with higher TMB. Patients with TMB over the median (>158 mutations) had a DCB of 65% versus 34% for those with lower TMB (P=0.011) (1).

These data have been recently confirmed in the prospective study Check-Mate 227, published in the *New England Journal of Medicine*, identifying that patients who benefit from immunotherapy (nivolumab plus ipilimumab) over chemotherapy at first line setting were those with high TMB ($PFS_{1y}$was 42.6% with nivolumab plus ipilimumab versus 13.2% with chemotherapy in TMB high, but differences were not significant in patients with TMB low) (2). Based on these results, Bristol-Myers Squibb has presented the supplemental Biologics License Application for the combination of nivolumab plus ipilimumab in NSCLC with high TMB in first line setting to the FDA. TMB is the quantification of the number of somatic mutations per genome area in a tumor. In 2013 the analysis of TMB in different tumor types demonstrated that NSCLC and melanoma usually carry high TMB compared to other tumor types, with 10–400 mutations per megabase (3).

Before Hellmann's study, other authors had suggested that tumors with high number of mutations were those that had more chance of responding to immunotherapy (4-6), leading to the hypothesis that quantifying the number of mutations carried by a specific tumor could help in patient selection.

Somatic mutations are produced by carcinogens, such as tobacco, or by intrinsic tumor alterations in genes that repair DNA abnormalities, such as DNA mismatch repair genes. Tumors that accumulate a higher number of mutations have higher chances of codifying peptide epitopes that can be identified by lymphocytes ("neoantigens") (7).

Since the techniques to identify tumor neoantigens are very complex (8), measuring the total number of mutations in a particular tumor can be an indirect and more simple way of evaluating the probability of expressing neoantigens. Hellman *et al.* demonstrated with his work that TMB, especially when it is calculated using only nonsynonymous variants, was not less predictive of clinical activity of nivolumab plus ipilimumab than computational predicted neoantigen burden (1), as others have demonstrated in urologic carcinomas (9). Helmann *et al.* also found a high correlation of TMB with neoantigen load (1), suggesting that the high TMB reflects an increased potential for

immunogenicity.

In the clinical setting, whole exome sequencing (WES) has several caveats. It is time, money and tissue consuming, and is still a laborious technique that is mainly used for research. Other more simple techniques that analyze a custom set of genes by next generation sequencing (NGS) are currently available in the clinical setting, such as the FoundationOne panel from Roche, that analyzes 315 genes, or the MSK-IMPACT panel (10). These platforms can estimate TMB just as accurately as WES, according to the Hellmann *et al.* work (1) and other studies that have used different NGS platforms (11-13). The cutoff value for defining "high TMB" with the FoundationOne panel was calculated in the phase II study Check-Mate 568 using response rate as endpoint. Patients with high TMB, defined as ≥10 mutations per megabase, had a response rate of >40% (14). This cutoff was used later in the phase III trial that has demonstrated benefit with the combination of nivolumab and ipilimumab over chemotherapy, only for patients with high TMB (3).

Nevertheless, negative predictive value of TMB is not perfect. In the Hellmann *et al.* study, 34% of patients with low TMB had DCB (1). It is well known that other factors are also associated to clinical benefit with immunotherapy. Indeed, some types of tumors, such as renal carcinoma or virus-associated Merkel cell carcinoma, have a high rate of durable responses (15,16) despite carrying low TMB. The identification of specific molecular alterations that predict response or resistance to immunotherapy has opened the door to more personalized ways of decision making for selecting therapies. Mutations in genes that encode components of the PBAF form of the SWI/SNF complex involved in chromatin remodeling, such as *PBRM1* (a gene codifying BAF180, that is frequently mutated in renal cell carcinoma) or in *ARID2* (frequently mutated in melanoma), are predictive of response to immunotherapy (15,17). Loss of function of PBAF produces higher secretion of chemokines in response to interferon gamma, which leads to a higher tumor T cell infiltration (18). Other mutations that have been identified as predictive of response are mutations in *PTPN2*, a protein tyrosine phosphatase that dephosphorylates JAK1 and STAT1, leading to suppression of interferon gamma signaling. Loss of *PTPN2* produces higher expression of MHC-I in response to Interferon gamma, as well as higher levels of antigens loaded to MHC-I (19). Several mutations have been identified as predictors of resistance to immunotherapy, such as, those affecting genes involved in the Interferon gamma pathway, as the gene encoding the ligand binding chain of the interferon gamma 1 receptor (*IFNGR1*, *CD119*) (19), Janus kinases (*JAK1* and *JAK2*) (12,19,20), beta-2-microglobulin (*B2M*) (21), and apelin receptor (*APLNR*, *APJ*) (18), as well as mutations in genes that regulate WNT/β catenin pathway, for example, the liver kinase B1 gene (*LKB1*, *STK11*) (22,23) and phosphatase and tensin homolog gene (*PTEN*) (24). The paper of Hellmann *et al.* also analyzes whether mutations in some of these individual genes have predictive value (on Table S4 from the commented paper). The *STK11* mutation was found in seven patients and *PTEN* mutation in four patients, all of them non-responders (1). Mutations in *IFNGR2*, *APLNR*, *PTPN2*, *CD274* (*PD-L1*) were found, just one case of each, all of them in non-responders. Contrary to expectations, mutations in *B2M* (in one patient) and in *IFNGR1* (in three patients) were found in responders (1). Mutations in common driver genes of NSCLC, such as *TP53* or *KRAS*, were slightly more frequent in responders than in non-responders (71% versus 45% for *TP53*, 38% versus 27% for *KRAS*), while *EGFR* mutations were more frequent in non-responders (18% versus 8%) (1).

In this paper the authors also analyze the correlation of TMB with PD-L1 expression (using a cutoff value of >1%), since PD-L1 is the only predictive biomarker used at the clinical setting for patient selection. Response rate was higher (62.5%) in patients who have high TMB and PD-L1 positive expression than in those with TMB high but PD-L1 negative (33.3%), TMB low and PD-L1 positive (14.3%) or TMB low and PD-L1 negative (7.7%) (1). A similar finding had been previously suggested from an exploratory analysis of the CheckMate-026 study comparing nivolumab versus chemotherapy in naïve NSCLC patients (6) and in a retrospective analysis of 240 patients treated with anti PD-1 or anti PD-L1 antibodies (10).

Even using the combination of PD-L1 expression and TMB, the negative predictive value continues to be incomplete, as one patient with low TMB and negative PD-L1 expression had an objective response (1). More recently, the same group published in the prospective phase III trial of nivolumab plus ipilimumab in patients with high TMB that PD-L1 expression did not add any predictive value to the analysis of TMB (2).

Other possible predictive factors of response, such as Interferon gamma expression (25), tumor microenvironment composition (26), T cell receptor clonality (27) or PD-1 expression (28), could add further information to a predictive model.

Although TMB analysis is "only" an indirect way for the estimation of the presence of neoantigens, it is feasible at the clinical setting and it identifies NSCLC patients more prone to respond to the nivolumab plus ipilimumab combination. Although high TMB does not identify all patients who will respond, combination with other predictive markers will help us in patient selection for immunotherapy.

Acknowledgements

None.

Footnote

Conflicts of Interest: Santiago Viteri received advisory honoraria and travel expenses paid by Roche; speaker honoraria paid by BMS. Maria Gonzalez-Cao received advisory honoraria paid by Pierre Fabre, BMS; travel expenses paid by Pierre Fabre, BMS, Roche; speaker honoraria paid by BMS, AZ. The other authors have no conflicts of interest to declare.

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Cite this article as: Gonzalez-Cao M, Viteri S, Karachaliou N, Aguilar A, García-Mosquera JJ, Rosell R. Tumor mutational burden as predictive factor of response to immunotherapy. Transl Lung Cancer Res 2018;7(Suppl 4):S358-S361. doi: 10.21037/tlcr.2018.10.03

