New insights in predictive determinants of the tumor immune microenvironment for immune checkpoint inhibition: a never ending story?

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Introduction

Checkpoint inhibitors such as Pembrolizumab (Keytruda®, MSD), Atezolizumab (Tecentriq®, Roche), Durvalumab (Imfinzi®, AstraZeneca) or Nivolumab (Opdivo®, BMS) have significantly improved curative and palliative treatment of solid malignancies (1-4). However, clinical responses vary largely across different tumor entities. Since the currently used standard predictive tool—PD-L1 assessment by immunohistochemistry—shows largely varying predictive efficacy in different tumor types, e.g., in urothelial cancer (5-7), there is a heavy need for additional complementary biomarkers which can improve the current immunotherapy selection and prediction of therapy success.

Recent results of the KEYNOTE-028

Recently published results from the KEYNOTE-028 study which investigated the clinical efficacy of Pembrolizumab in patients with PD-L1 positive advanced solid malignancies revealed new insights in immune-oncological determinants for immunotherapy responsiveness (8). This basket trial consists of patients suffering from multiple solid malignancies beyond the big entities where immune checkpoint inhibition is already standard of care in many indications. The investigators applied a variety of different molecular analysis in particular analysis of a previously published T-cell-inflammation related gene expression signature consisting of 18 genes (9,10), PD-L1 assessment using the 22c3 pharmDx assay for combined positive scoring (CPS) (11), and TMB analysis using a common NGS platform (8). The investigators found that patients out of this basket trial had improved objective response rates in case of elevated expression values as detected by the 18-gene gene expression signature, high PD-L1 expression and high TMB. Since these parameters showed only weak inter-marker correlations each single biomarker was able to exclusively identify patients who benefitted from anti-PD-1 targeted treatment (8). These findings build optimism that complementary biomarkers beyond PD-L1 IHC assessment could identify additional patients profiting from immune checkpoint inhibition. If a sequential testing of PD-L1, the inflammatory milieu (e.g., by gene expression signatures) and TMB might be an option for the clinical practice has to be investigated in upcoming studies. However, these results once more indicate that one biomarker alone will not be sufficient for a proper selection of patients who would benefit from immunotherapy, especially not in the setting where immune checkpoint inhibition is no current standard of care e.g., in sarcomas, tumors of the salivary glands,

neuroendocrine tumors or other rare entities which were part of the KEYNOTE-028 (8).

Current state of IO biomarker testing

The immunotherapeutic field in oncology is currently one of the most dynamic research areas. Drugs targeting PD-1, its ligand PD-L1 or CTLA-4 revolutionized the field of anti-cancer treatment of several solid malignancies such as melanoma, NSCLC or urothelial carcinoma (2,4,12). In the particular case of urothelial carcinoma, after a long void of over 40 years, checkpoint inhibitors were the first effective therapy option in platinum-refractory metastatic urothelial carcinoma patients (13,14).

Despite the undeniable therapeutic advances through checkpoint inhibition there are several critical aspects which need to be discussed. At the moment, PD-L1 testing is current standard of predictive testing in several indications in NSCLC, head and neck squamous cell carcinoma or urothelial carcinoma (15-17). One important example for the current PD-L1 "misery" is the predictive role of PD-L1 in urothelial cancer: Recently, the FDA and EMA restricted the use of Keytruda (Pembrolizumab) and Tecentriq (Atezolizumab) in urothelial carcinoma based on still unpublished interim data (15), although PD-L1 testing was previously not indicated in other approved indications due to low correlation of PD-L1 status with progression free and overall survival benefits of immune checkpoint inhibition (2,6,13,14). Reasons for this "consistent inconsistency" could be numerous, but important issues lay in the nature of immunohistochemical PD-L1 assessment: although recent studies suggested that all approved companion diagnostic assay could be used more or less interchangeably for immune cell scoring and for tumor cell scoring with the exception of the SP142 assay, which detects significantly lower amounts of tumor cells, the inter-algorithm variability remains (7,18). Our group could show that the now prescribed algorithms for Atezolizumab (5%-IC-score) and Pembrolizumab (CPS10) identify different patient populations (7): of 125 patients which were positive for at least one of the two scoring systems only 41.6% were diagnosed as eligible for both drugs, while the other 58.4% were only eligible for one of the both drugs. Assuming that different PD-1/PD-L1 targeting drugs show comparable efficacy, this inter-algorithm variability could explain at least a part of the inconsistent predictive value of PD-L1 IHC scoring with different algorithms. Further issues with PD-L1 testing lay in the lack of trained pathologists which are aware of all relevant PD-L1 algorithms for different indications as well as reimbursement and supply issues in several countries. Since all approved companion diagnostic assays are quite expensive, many health insurances—especially in Europe—are not reimbursing those tests. Amongst this, the platform dependency of Dako (Dako 22c3, Dako 28-8; Dako autostainer dependent) and Ventana assays (Ventana SP142, Ventana SP263; Ventana autostainer dependent) led to the widespread use of lab developed tests most based on freely available antibody clones such as the E1L3N (CellSignaling), 28-8 (Dako) or 22c3 (Dako) which might lack sufficient validation (19,20). Furthermore, inter-observer variability could also lead to significant issues in proper patient selection which could be prevented by widespread and systematic training of pathologists for important scoring algorithms for different indications (21). Large-scale harmonization trials led to substantial improvements of PD-L1 assessment in NSCLC (22), and are therefore heavily needed for other entities such as urothelial carcinoma or head and neck squamous cell carcinomas.

Among the "PD-L1 testing misery", there are further promising, more objective biomarkers for predicting improved outcomes under immune checkpoint inhibition such as the deficiency of DNA mismatch repair genes which causes the truncation of the subsequent DNA repair proteins (23,24). The most prominent genetic alteration linked with these important mechanisms to maintain the genomic integrity of normal cells, are the four central DNA mismatch repair proteins MSH2, MSH6, MLH1 and PMS2. Deficiency of one of these four genes is causing classical microsatellite instability (MSI)—either sporadic or as germline variant (Lynch-syndrome) (25). In the pre-NGS era, MSI was considered to occur more or less exclusively in patients with Lynch-syndrome which most often develop colorectal malignancies, but recent large-scale tumor genetic analysis showed MSI to be more frequent as sporadic event in multiple cancer types than previously thought (26). Due to the impaired DNA repair, MSI-H tumors are considered to achieve numerous non-synonymous somatic mutations which lead to an increased neoantigen burden (26). Recent studies clearly demonstrated that patients suffering from MSI-H malignancies—irrespective of primary localization and sporadic or hereditary occurrence—are benefiting from checkpoint inhibitors (27-31). Approval of pembrolizumab for MSI-H malignancies represented the first agnostic histology approval and set the stage for biomarker-based drug approvals. Patients with different solid tumors

achieving high non-synonymous tumor mutational burden due to other genetic alterations causing genetic instability have also been shown to benefit from anti-PD-1/PD-L1 targeted immunotherapy. At the moment this has been proven for e.g., NSCLC, melanoma and also urothelial carcinoma (32-36). As highlighted once more by the Keynote-028 other immunologic determinants such as T-cell inflammation related gene expression correlates with improved response to checkpoint inhibition in several solid tumors (9).

Beside those promising biomarker strategies other concepts are currently investigated to further improve immunotherapy of solid tumors: TGF-β is a cytokine connected with several pro-tumorigenic effects such as promoting immunosuppression, angiogenesis, fibroblast activation and metastasis by actively excluding immune cells from the tumor mass due to fibroblast induced expansion of the extracellular matrix (34). Anti-TGF-β targeted antibodies were previously shown to potentiate the anti-tumoral activity of Atezolizumab in a murine model by reprogramming fibroblasts and increasing the amounts of $CD8^+$ T-cells in the tumor mass (34). The benefit of additional TGF-beta blockade is currently investigated in several ongoing clinical trials.

Conclusions

Taken together, the current era of immunotherapy in solid tumors is an exciting and rapidly changing field. Although the therapeutic effects are undeniable it will be absolutely necessary to develop new diagnostic strategies beyond "simple" PD-L1 IHC assessment to identify patients who are truly benefitting from immune checkpoint inhibition while patients who won't benefit should rather undergo other targeted therapies if possible. Since biomarkers like PD-L1, immune cell infiltration, TMB or MSI-status are not always correlated a sequential or parallel panel testing of these markers could potentially improve a proper patient selection for immunotherapy.

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Footnote

Conflicts of Interest: S Gupta is an advisor for Merck, Pfizer, BMS. Has received honorarium from Exelixis and Janssen; M Eckstein is advisor for AstraZeneca and Janssen-Cilag; received speaker's honoraria from AstraZeneca, Roche, Astellas and Janssen-Cilag.

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