Introduction

Deficient DNA mismatch repair (MMR) results in a hypermutated phenotype characterized by microsatellite instability (MSI) and a higher burden of mutation-associated neoantigens that are targeted by the immune system. Deficient MMR and high frequency MSI (MSI-H) have been shown to be predictive biomarkers for immune checkpoint inhibitor drugs which block the programmed death protein-1/programmed death ligand-1 (PD-1/PD-L1) interaction between tumor cells and activated T cells. These agents include antibodies against PD-1 and PD-L1 that have significantly altered the treatment algorithm for several solid tumors. Presence and level of PD-L1 tumor expression are associated with responsiveness to these antibodies in certain malignancies that include advanced melanoma, non-small cell lung cancer, renal cell carcinoma, urothelial cancer and Hodgkin’s lymphoma.

In 2017, the US Food and Drug Administration (FDA) granted accelerated approval to pembrolizumab (KEYTRUDA) for patients with unresectable or metastatic d-MMR solid tumors based upon an assessment of 15 cancer types, among which, 149 MSI-H patients were enrolled across 5 clinical trials [1]. A complete or partial response was experienced by 39.6% of patients, with responses lasting >6 months in 78%. Based upon these data, the FDA approved a cancer treatment for the first time based upon tumor biomarker status, rather than a specific disease-based approach.

Vanderwalde and colleagues recently published their experience evaluating a broad spectrum of 11,348 solid tumors noting the frequency of dMMR based on having MSI-H: PD-L1 expression to potentially guide the delivery of tumor agnostic immunotherapy.

EUS fine-needle pancreatic core biopsy can determine eligibility for tumor-agnostic immunotherapy

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ABSTRACT

Background and study aims The US FDA recently approved a cancer treatment with pembrolizumab based upon the tumor biomarker status of deficient mismatch repair (dMMR) rather than a specific disease-based approach. We sought to determine if endoscopic ultrasound-guided fine-needle biopsy (EUS-FNB) could determine dMMR and quantification of PD-L1 expression to potentially guide the delivery of tumor agnostic immunotherapy.

Patients and methods Immunohistochemistry was performed on archived pancreas core biopsy specimens. Tumors with absent nuclear staining of DNA mismatch repair proteins represented dMMR. Tumors were considered to have any or high PD-L1 expression, if expressed in ≥1% or ≥50% of tumor cells.

Results Histologic specimen adequacy for MMR status assessment was satisfactory in 97.2% of tumors. dMMR and high PD-L1 expression was identified in 3% and 8.1% of the cohort.

Conclusion In the setting of tumor type agnostic immunotherapy, it is projected that at least 3% of malignant pancreas lesions will be sensitive to pembrolizumab and up to 8% sensitive to the family of immune checkpoint inhibitors. This highlights the expanding role of EUS-FNB in the field of precision immuno-oncology.
pression in endometrial cancer (17%: 16.2%), gastric adenocarcinoma (8.7%: 18.5%), colorectal adenocarcinoma (5.7%: 7.2%), cholangiocarcinoma (2.3%: 18.6%), pancreatic ductal adenocarcinoma (PDAC) (1.2%: 21.6%), renal cell carcinoma (0.6%: 29.7%) and melanoma (0%: 42.3%) [2]. The fact that dMMR is rarely present among PDAC patients was further demonstrated in a study of 833 surgically resected PDAC tumors revealing a frequency of 0.8%, all of whom were patients found to have Lynch syndrome [3].

In an era of evolving tumor agnostic immunotherapy, we questioned if pancreatic endoscopic ultrasound-guided fine-needle biopsy (EUS FNB) provides sufficient material for dual immunohistochemistry (IHC) for dMMR and PD-L1 status evaluations. We sought to determine the prevalence of MSI-H status and quantification of PD-L1 expression to determine their utility in guiding disease-agnostic immunotherapy based upon biomarker status.

Patients and methods

IHC was performed on consecutive archived treatment-naïve formalin-fixed paraffin-embedded EUS pancreas core biopsy specimens (2001 – 2017 IRB # 17-006528). Briefly, 4-µm-thick tissue sections were stained using the Ventana BenchMark XT automated slide-staining system using the following antibodies: Anti-PD-L1 (clone SP263, VENTANA, Tucson, AZ), MLH1 (clone G168-728, Cell Marque, Rocklin, California, United States), MSH2 (clone FE11, Biocare Medical, Concord, Massachusetts, United States), MSH6 (clone BC/44, Biocare Medical, Concord, Massachusetts, United States), and PMS2 (clone A16-4, Biocare Medical, Concord, M Massachusetts, United States). Antigen-antibody reactions were visualized using UltraView detection with diaminobenzidine as the chromogen. A minimum of 100 viable tumor cells were required on one stained slide for the specimen to be considered adequate for evaluation. Positive PD-L1 expression was defined as membranous staining. The approximate percentage of positive tumor cells versus all tumor cells provided the Tumor Proportion Score (TPS). The specimen was considered to have PD-L1 expression if PD-L1 was expressed in ≥1% of tumor cells and a high level of expression if ≥50%. Tumors were classified as dMMR if they exhibited absent nuclear staining of DNA mismatch repair proteins (MLH1, MSH2, MSH6, or PMS2).

Results

Clinical demographics

Thirty-nine treatment-naïve patients with either a primary or secondary pancreas malignancy who underwent EUS with FNB were assessed for histologic specimen adequacy for MMR and PD-L1 expression status. Age of the overall cohort was 71.2 ± 10.2 years, 61.5% were male, and the overall mortality rate was 25.6% at 7 months (4.9–15.7) following EUS diagnosis. The study cohort was composed of 21 patients with PDAC who had previously reported PD-L1 expression analysis and with patient details as follows: 72.9 ± 8.9 years; 57% male; CA19-9 level = 143 U/mL (18 – 998); 57% 8th AJCC Stage ≥ III) [4]. The study also included 18 patients (69.3 ± 11.6 years; 66% male) with either a renal cell cancer (RCC) (n = 16) or melanoma that had metastasized to the pancreas (n = 2). Using either a 19G Trucut biopsy needle (n = 9 patients) (Quick-Core Biopsy Needle, Cook Medical, Winston-Salem, North Carolina, United States) or a 22G Sharkcore FNB needle (n = 30 patients) (SharkCore FNB needle, Medtronic, Dublin, Ireland), tumor tissue was sampled with an overall median tissue aggregate that was 19 mm (12 – 20) in length following 2 (3–5) EUS-FNB passes.

DNA mismatch repair status

Among the 39 patients, 36 had specimen blocks available for retrieval from the tissue archive and 35 (97.2%) patients (FNB passes 2 (3–5)) had histologic specimen adequacy to assess dMMR by IHC. One patient (2.9%) displayed loss of MLH1-PMS2 (Fig.1). The patient was a 64-year-old male with decompensated cirrhosis secondary to nonalcoholic steatohepatitis, a splenic artery pseudoaneurysm resulting in hemosuccus pancreaticus and previously resected colon cancer. He presented with cholestatic jaundice and an elevated CA19-9 (1,184 U/mL) as a consequence of a 4-cm EUS T4N0M0 PDAC head mass. The additional immunostains performed on the tumor showed that the tumor cells were positive for CK7 and focally positive for CDX2 and CK20. This immunophenotype supports the diagnosis of a primary PDAC. He was started on FOLFRINOX. He subsequently underwent germline testing which detected Lynch syndrome and received pembrolizumab. He had stable disease on therapy, but succumbed to vascular complications, presumably from his cirrhosis, 7.7 months following EUS.

MMR status and PD-L1 expression

Thirty-seven patients (94.9%) had specimens that were satisfactory for PD-L1 expression assessment (Fig. 2). Expression thresholds of ≥ 1%, ≥ 5%, ≥ 10%, ≥ 25% and ≥ 50% expression in tumor cells were identified in 16 (43.2%), 13 (35.1%), 7 (18.9%), 5 (13.5%) and 3 (8.1%) patients, respectively (Fig. 3). Tumor tissue from 33 patients underwent dual MSI and PD-L1 immunostaining (Fig. 4). PD-L1 expression (> 1%) with proficient (p) MMR or dMMR status was identified in 13 (39.4%) and 1 (3%) patient, respectively. The tumor from the patient with Lynch syndrome had PD-L1 expression in 10% of tumor cells.

Biomarker-based immunotherapy eligibility

Based upon the prevalence of dMMR status, 3% of our patients would be eligible to receive pembrolizumab in the setting of a FDA-approved disease-agnostic immunotherapy approach. PD-L1 expression was high (TPS > 50%) in 8.1% of patients, suggesting that expression of this biomarker by their tumors may indicate potential sensitivity to immune checkpoint inhibitor therapy.

Discussion

Among a unique patient cohort, we determined that a paired evaluation of MMR status and PD-L1 expression by IHC is possible using specimens obtained with EUS-FNB. Our findings de-
▶ Fig. 1a–e Loss of MLH1-PMS2 protein expression in a PDAC EUS-FNB specimen.

▶ Fig. 2 a EUS-FNB malignant melanoma metastasis to the pancreas with b PD-L1 expression > 90%.
Produce the capability of using tissues obtained with EUS-FNB for molecular classification of treatment naïve solid pancreas masses by identifying biomarkers that may guide use of checkpoint inhibitor therapy. The prevalence of dMMR in our cohort indicates that patients with these tumors are candidates for treatment with pembrolizumab. Furthermore, 8.1% of evaluated tumors had a high level of expression of PD-L1 (TPS >50%), which in certain tumor types is associated with responsiveness to checkpoint inhibitor therapy.

Following much debate, it has been established that endoscopic ultrasound-guided fine-needle aspiration and EUS-FNB needles yield a similar satisfactory specimen for diagnostic purposes, with the caveat that fewer FNB passes are required to achieve such a yield [5–10]. However, when tissue for companion diagnostic or prognostic tumor information is required for integration of molecular analyses into clinical decision-making, the optimal needle landscape changes depending upon the specific objective. Options for use of tissue specimens include IHC, genotyping using next-generation sequencing (NGS), or establishing tumor organoids in a research setting. PDACs demonstrate only 5% to 20% neoplastic cellularity and are characterized by a prominent desmoplastic reaction considered to be a hostile tumor microenvironment (TME) for subsequent therapy [11,12]. Cytology smears are thought to be superior for multiplex gene panel NGS evaluations, as their overall cellularity, tumor fraction, and sequencing metrics are considered to be superior to that of FNB, yet, successful preclinical disease models to include organoid development require that EUS-FNB deliver tumor cells and the corresponding TME [13–15].

As endosonographers, while we try to navigate the provision of satisfactory material for ancillary molecular analysis to improve cytologic adequacy and identify predictors of prognosis and chemosensitivity, we are now poised to assist in patient selection for precision immuno-oncology. There are a number of barriers to the utility of immunotherapy in PDAC as the mutational burden is low; the TME is characterized by dense stroma with very few T cells, collectively creating a “cold” or non-immunogenic environment and potentially making such tumors less responsive to checkpoint inhibitors. Yet, there is much need for enhanced tissue access and sampling for analysis to utilize biomarkers in patients with solid tumors, including PDAC, which can determine eligibility for checkpoint inhibitor therapy.

To date, the MMR/MSI status of pancreatic lesions has only been evaluated in surgically resected specimens, which represent a small proportion of patients that have earlier, less advanced disease, as patients with more advanced disease are no longer surgical candidates. MMR and PD-L1 status may be influenced by prior neoadjuvant or adjuvant therapy as has been observed in both breast and colon cancer analyses [16,17]. Therefore, for primary pancreatic masses to include PDAC, it may be more desirable to perform such analysis on treatment naïve specimens for which EUS-FNB is ideally suited to establish the molecular signature. For secondary pancreatic metastasis, we evaluated RCC and melanoma treatment naïve lesions, which is the ideal scenario. However, from a practical perspective, patients with these lesions have usually undergone prior treatment that requires careful consideration of the molecular testing.

**Conclusion**

In conclusion, we highlight that our early experience with dual MMR IHC and PD-L1 expression analysis shows that it is technically feasible to assess biomarker-based immunotherapy eligibility with EUS-FNB specimens from solid primary and secondary pancreatic masses. Based upon the prevalence of dMMR and high PD-L1 expression, 3% of the treatment naïve cohort is a candidate for checkpoint inhibitor therapy and high PD-L1 expression may provide additional information regarding sensitivity to immunotherapy depending upon tumor type.

**Competing interests**

None

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**Fig. 3** Patient prevalence and quantification of PD-L1 expression positivity for EUS-FNB specimens of treatment naïve PDAC, clear cell renal cell carcinoma and malignant melanoma.

**Fig. 4** Dual MSI and PD-L1 IHC assessment yield.

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