Neuroendocrine tumors (NETs) and neuroendocrine carcinomas (NECs) are a diverse family of neoplasms that range in behavior from indolent to highly aggressive. Well-differentiated NETs frequently originate from enterochromaffin cells in the gastrointestinal tract and lungs. They are characterized clinically by a relatively slow growth rate (compared to most malignancies) and a propensity to produce hormones and vasoactive substances (1). The genetic landscape of well-differentiated NETs typically consists of mutations in genes such as MEN1 and DAXX, which are associated with chromatin remodeling (2,3). Tumor mutational burden is relatively low, and microsatellite instability is extremely rare (4,5). Poorly-differentiated NECs are highly aggressive malignancies, typically categorized as small cell and large cell. Small cell lung carcinomas and Merkel cell cancers are variants of poorly differentiated NECs which fall outside of the scope of this article. The mutational landscape of poorly differentiated NECs is similar to that of non-neuroendocrine cancers, with mutations in p53 and Rb1 predominating. Tumor mutational burden is generally higher than observed in well-differentiated NETs (5-7).

Clinical trials of immunotherapy have only recently been completed in neuroendocrine neoplasms (NENs). Although data have demonstrated a relatively limited role for PD-1 inhibitor monotherapy, other immunotherapeutic approaches may yield improved results. In this article, we summarize the preclinical data on the immune landscape of NETs and NECs, and review the key clinical trials conducted thus far.
The immune landscape of NENs

Multiple immune cell types, including T cells, NK cells, mast cells, macrophages as well as dendritic cells infiltrate NENs of different origins and grades. However, despite tumor infiltration and possibly immune recognition, NENs are able to escape the host immune response and avoid immunosurveillance by exploiting multiple local and systemic resistance mechanisms including the deactivation of T cells, dysregulation of T regulatory (Treg) cells and the creation of an immunosuppressive cytokine milieu with tolerogenic properties.

Lymphocyte infiltration—pNETs

Lymphocyte infiltration is a frequent event in both gastroenteropancreatic (GEP) and bronchial NETs. In a series of 87 pancreatic NETs (pNETs), CD3+ T cell infiltration was reported in 68% of the tumors and was not associated with tumor grade or other clinicopathological variables. Among patients with intermediate-grade pNETs, low-density lymphocyte infiltration appeared to predict recurrence following tumor resection compared to high density infiltration (8). Conversely, in a recent study of 244 GEP-NETs, high levels of intratumor lymphocyte infiltration were described to be significantly associated with higher tumor grade and shorter survival (9). Consistently, in a multispectral imaging analysis comparing 47 low-grade pNETs with 5 high-grade pNETs and pNECs, T cell infiltration increased with grade (10).

Lymphocyte infiltration—small bowel NETs

In a cohort of 102 G1/G2 primary small bowel NETs, an intratumor host immune response was reported in approximately two-thirds of tumors, with the extent of the lymphocyte infiltration being significantly higher in duodenal NETs as compared with jejunal or ileal NETs (11). Of note, ectopic lymph nodes with activated germinal centers were observed at the tumor edge in about one-fifth of the cases. In another study of 62 patients with small bowel NETs, lymphoid aggregates were found in 27% of tumors, and infiltration of CD8+ T cells was described in the 97% of the samples (12). At present, the biological significance of tertiary lymphoid structures in NETs remains unclear. Intriguingly, T lymphocytes have been reported to specifically recognize NET cells. Indeed, the presence of CD8+ T cells reactive against NET-associated antigens such as chromogranin A or tryptophan hydroxylase has been demonstrated in patients with midgut NET (13). More recently, T cells reactive against tumor neoantigens have been recognized in the blood of patients with metastatic rectal NETs (14).

Treg-driven immunosuppression

The presence of immunosuppressive FoxP3+ Treg cells has been shown to be more abundant in high- versus low-grade pNETs, and independently predicts dismal prognosis (8,19). Regardless of the density of tumor infiltration, circulating levels of Treg cells have been found to be significantly higher in patients with midgut NETs as compared with healthy subjects, and the lower proliferative capability of T cells derived from patients with midgut NETs has been ascribed to a Treg-driven suppression of systemic Th1-promoting cytokines such as IL-12 and IL-1b (20).

NK cells have demonstrated impaired cytolytic activity in GEP-NETs. In particular, a deficient interferon (IFN)-a response has been observed in patients with midgut NETs, where NK cell activity could be restored by exogenous treatment with interferon (21). Moreover, an increased NK cell activity has been associated with tumor regression (22). Mast cells may have a prominent role in pNET progression. Evidence from a mouse model of pancreatic b-cell tumorigenesis suggests that tumor-infiltrating mast cells regulate neoangiogenesis and tumor expansion.
In this context, pharmacological inhibition of mast cell degranulation has proved effective in inducing cancer regression in mice harboring islet-cell tumors (23,24).

**Tumor-infiltrating macrophages and antigen presentation**

Evidence from murine models suggests that tumor-infiltrating macrophages contribute to both angiogenic switch and pNET progression (25). Consistently, the density of macrophage infiltration appears to be higher in poorly differentiated NECs than in well-differentiated NETs (26). Large series studies have shown that a dense macrophage infiltration predicts recurrence following surgery (27,28).

Antigen presentation is potentially impaired in NETs. Carcinoid-specific soluble immune inhibitory factors have been shown to down-regulate both maturation and function of dendritic cells in bronchial NETs (29). In addition, in a study of 104 surgically resected pNETs, the expression of HLA class I molecules has been demonstrated to be defective in 70% of cases (28). In another study, the MHC molecule b2-microglobulin has been shown to be altered in 10/11 samples of pNETs (30).

**Immune checkpoint inhibition**

In recent years, multiple investigations have been carried out to characterize the expression of the immune checkpoint molecules programmed death-ligand 1 (PD-L1) and programmed death-1 (PD-1) in NETs and NECs (9,11,17,18,28,31–41). As shown in Table 1, both the expression of PD-L1 and the extent of tumor infiltration by PD-1 lymphocytes appear to be higher in high-grade or poorly differentiated neoplasms rather than in well-differentiated tumors. Differences in the clinical characteristics of accrued patients (i.e., primary site, grade, fraction of metastatic cases), in the type of samples analyzed, in the mAb clone used for PD-L1 testing as well as in the criteria used for staining interpretation may account, at least in part, for the heterogeneity of results seen across different studies. It is currently unclear whether the expression of PD-1 or PD-L1 has any prognostic potential.

**Clinical trials of immune checkpoint inhibitors**

Several phase II studies have recently explored single-agent and combination therapy with immune checkpoint inhibitors.

**Monotherapy trials**

The KEYNOTE-028 study, a large multi-cohort phase 1b study evaluating the safety and efficacy of pembrolizumab in patients with PD-L1-positive advanced solid tumors included 41 NET patients (42). Four (10%) patients experienced objective responses while 71% experienced stable disease. Duration of response ranged from 6.9–17.6 months in the 4 responders. This data led to the inclusion of a NET cohort on the subsequent KEYNOTE-158 study. The KEYNOTE-158 study included a large cohort of “well and moderately differentiated” NETs originating in the lung, appendix, small intestine, colon, rectum, or pancreas (43). Patients were required to have progressed on at least one prior line of therapy, with no limit on the number of prior lines. Therapy consisted of pembrolizumab at a standard dose of 200 mg every 3 weeks, for up to 2 years. The primary endpoint was overall response rate (ORR), assessed per RECIST 1.1 by independent central radiology review. Of the 107 patients who were treated on the NET cohort, 67.3% had received ≥2 prior therapies and 15.9% had PD-L1 positive tumors [defined as combined positive score (CPS) ≥1 on IHC analysis]. At the time of data cut-off, ORR was 3.7% (95% CI, 1.0–9.3%), with 4 partial responses (PR) and no complete response (CR). Of the four patients with PRs, three had pancreatic, and 1 had a gastrointestinal NET of unknown primary, all of whom had PD-L1 negative tumors. PFS was 4.1 months (95% CI, 3.5–5.4 months) and the 6-month PFS rate was 38.2%. Median overall survival (OS) was not reached at the time of data cut-off, and the 6-month OS rate was 84.6%. Treatment-related adverse events (AEs) occurred in 75.7% of patients, with 20.6% having grade 3-4 AEs.

Another phase II study of pembrolizumab was conducted in high-grade NENs, excluding NENs of thymic or lung origin, who had progressed on prior platinum-based therapy (44). Therapy consisted of pembrolizumab at a standard dose of 200 mg every 3 weeks, for up to 2 years. The primary endpoint was ORR per RECIST 1.1. Of the 21 patients enrolled, 15 patients had available archival tissue for PD-L1 and tumor infiltrating lymphocytes (TILs) testing. Forty-seven percent had P-L1 staining >1% and 53% had evidence of TILs >2+ (>10 TILs/HPF). At the time of data cut-off, 16 patients were evaluable for response. ORR was 4.7%, with one PR and no CRs. Of the remaining patients, 3 (4.7%) patients experienced SD and 12 (57%) experienced PD. The one patient with a PR
Table 1 The expression of PD-L1 and PD-1 in GEP and lung NENs: an overview

<table>
<thead>
<tr>
<th>Tumor location</th>
<th>N of samples (tumor grade)</th>
<th>% of metastatic cases</th>
<th>Anti-PD-L1 mAb used</th>
<th>Anti-PD-1 mAb used</th>
<th>Cut-off used for interpretation of positive staining</th>
<th>% of positive samples</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>GEP-NETs: 14 pancreas; 8 colon-rectum; 7 biliary tract; 2 duodenum; 1 stomach</td>
<td>32 (15 G2; 17 G3)</td>
<td>100%</td>
<td>Clone SP142</td>
<td>NP</td>
<td>PD-L1: ≥1% of tumor cells</td>
<td>PD-L1: 22% (41% in G3 tumors)</td>
<td>(31)</td>
</tr>
<tr>
<td>GEP-NETs: 48 small intestine; 62 pancreas</td>
<td>116 (66 G1, 34 G2, 4 G3, 6 unknown)</td>
<td>36%</td>
<td>Clone SP142; Clone 28-8; Clone 22C3</td>
<td>Clone NAT105</td>
<td>PD-L1: ≥5% of tumor cells; PD-1: ≥5% of TILs</td>
<td>PD-L1: 6% (clone SP142); 0% (clones 28-8 or 22C3); PD-1: 1%</td>
<td>(32)</td>
</tr>
<tr>
<td>GEP-NETs: 128 small intestine; 72 pancreas; 26 colon-rectum; 17 stomach; 1 biliary tract</td>
<td>244* (141 G1, 83 G2, 20 G3)</td>
<td>43%</td>
<td>Clone E1L3N</td>
<td>Clone NAT105</td>
<td>PD-L1: ≥1% of tumor cells; PD-1: ≥3 TILs per TMA punch</td>
<td>PD-L1: 9% (8% in G1/G2 tumors, 17% in G3 tumors); PD-1: 16% (14% in G1/G2 tumors; 54% in G3 tumors)</td>
<td>(9)</td>
</tr>
<tr>
<td>GEP-NETs: 16 small bowel; 10 pancreas; 10 stomach; 10 liver; 7 colon; 2 biliary tract; 2 others</td>
<td>57 (39 G1; 9 G2; 9 G3)</td>
<td>4%</td>
<td>Clone E1L3N</td>
<td>NP</td>
<td>PD-L1: 10% of tumor cells</td>
<td>PD-L1: 28% (0% in G1 tumors; 78% in G2 tumors; 100% in G3 tumors)</td>
<td>(33)</td>
</tr>
<tr>
<td>GEP-NETs: 64 small bowel; 31 pancreatic</td>
<td>95 (49 G1; 42 G2; 4 G3)</td>
<td>4%</td>
<td>Clone 9A11</td>
<td>Clone EH33</td>
<td>PD-L1: ≥5% of tumor cells; PD-1: ≥1% of immune cells</td>
<td>PD-L1: 0% in SI-NETs; 7% in pNETs; PD-1: 2% in SI-NETs; 0% in pNETs</td>
<td>(34)</td>
</tr>
<tr>
<td>pNETs</td>
<td>117 unknown</td>
<td></td>
<td>Clone E1L3N</td>
<td>NP</td>
<td>PD-L1: ≥5% of tumor cells</td>
<td>PD-L1: 42%</td>
<td>(35)</td>
</tr>
<tr>
<td>pNETs</td>
<td>70</td>
<td>68%</td>
<td>Clone SP142</td>
<td>NP</td>
<td>PD-L1: ≥5% or ≥1% of tumor cells</td>
<td>PD-L1: 3% (≥5% cut-off), 11% (≥1% cut-off)</td>
<td>(36)</td>
</tr>
<tr>
<td>pNETs</td>
<td>104 (57 G1; 47 G2)</td>
<td>0%</td>
<td>Clone E1L3N</td>
<td>NP</td>
<td>PD-L1: ≥5% of tumor cells</td>
<td>PD-L1: 53%</td>
<td>(28)</td>
</tr>
<tr>
<td>pNETs</td>
<td>102 (94 G1; 8 G2)</td>
<td>54%</td>
<td>Clone 28-8</td>
<td>Clone NAT105</td>
<td>PD-L1: ≥1%, 5% or ≥50% of tumor cells; PD-1: ≥1 TIL/5HPF</td>
<td>PD-L1: 59%; PD-1: 51%; PD-1: 39% (≥1% and ≥5% cut-off); 14% (≥50% cut-off)</td>
<td>(11)</td>
</tr>
<tr>
<td>SI-NETs: 88 small bowel; 10 duodenum; 3 unknown</td>
<td>70 (47 G1; 23 G2)</td>
<td>55%</td>
<td>Clone E1L3N</td>
<td>Clone NAT105</td>
<td>PD-L1: ≥5% of tumor cells; PD-1: any expression in TILs</td>
<td>PD-L1: 13%; PD-1: 23%</td>
<td>(12)</td>
</tr>
<tr>
<td>SI-NETs: 89 small bowel; 10 duodenum; 3 unknown</td>
<td>80 (22 G1/G2; 58 G3)</td>
<td>41%</td>
<td>Clone 28-8</td>
<td>Clone EPR4877(2)</td>
<td>PD-L1: ≥5% of tumor cells; PD-1: ≥5% of TILs</td>
<td>PD-L1: 59%; PD-1: 51%</td>
<td>(37)</td>
</tr>
<tr>
<td>SI-NETs: 168 (131 G1; 37 G2)</td>
<td>168 (131 G1; 37 G2)</td>
<td>7%</td>
<td>Clone E1L3N</td>
<td>Clone SP269</td>
<td>PD-L1: ≥1% of tumor cells</td>
<td>PD-L1: 5%; PD-1: 40%</td>
<td>(17)</td>
</tr>
<tr>
<td>SI-NETs: 105 (14 G1; 6 G2; 67 SCLC; 18 LCNEC)</td>
<td>105 (14 G1; 6 G2; 67 SCLC; 18 LCNEC)</td>
<td>0%</td>
<td>Clone E1L3N</td>
<td>NP</td>
<td>Not reported</td>
<td>PD-L1: 15% in G1, 20% in G2, 26% in SCLC, 50% in LCNEC</td>
<td>(38)</td>
</tr>
</tbody>
</table>

Table 1 (Continued)
was negative for PD-L1 and had evidence of >20 TILs/HPF. Median PFS was 9.14 weeks (95% CI, 6.71–13.14 weeks) and median OS was 15.4 weeks (95% CI, 13 weeks–not reached). Treatment-related AEs occurred in 37% of patients, with 28% having grade 3 AEs.

Two similar phase II studies were conducted in patients with high-grade NENs who had progressed on prior platinum-based therapy, one utilizing avelumab in NENs of any primary origin excluding small cell lung cancer and Merkel cell carcinoma, and another with pembrolizumab in extrapulmonary NECs (excluding well-differentiated grade 3 NENs) (45,46). Twenty-nine patients were treated with avelumab, and at time of data cut-off, median DCR after 8 weeks of treatment was 32% with 2 PRs (7%), and median OS was 4.2 months. Treatment related AEs occurred in 38% of patients, with 4% having grade 3 AEs. The pembrolizumab trial was designed as a 2-stage study, with patients enrolled on stage 1 receiving pembrolizumab monotherapy. Data from stage 1 of the study was recently presented, reporting that of 14 patients enrolled, ORR was 7%. Median PFS was 58 days and 43% of patients discontinued treatment for clinical deterioration or radiographic PD prior to the first scheduled scan at 9 weeks. At last follow-up, one patient was still on treatment after 19 cycles. Treatment related AEs were mild, with no patients experiencing grade 3-5 AEs attributable to therapy.

A phase II study of spartalizumab (PDR001), a humanized anti-PD-1 antibody, was conducted in patients with non-functional, well and poorly-differentiated NENs (47). Patients with a well-differentiated NET of GEP or thoracic origin, refractory to prior anti-cancer therapies, including everolimus, or poorly differentiated GEP NEC patients who progressed on at least one prior line of cytotoxic chemotherapy were eligible for enrollment. Patients were enrolled regardless of PD-L1 expression. The primary endpoint was ORR, assessed per RECIST 1.1 by independent central radiology review. ORR was 7.4% in well-differentiated NETs and 4.8% in poorly-differentiated GEP NECs. Patients with lung NETs had a higher ORR at 20%, although 2 of 6 responding patients expired shortly after initial response. Among patients with poorly-differentiated GEP-NECs, well-differentiated GEP-NETs and lung NETs, the rate of expression of PD-L1 in immune cells was 42%, 23% and 15% respectively. Biomarker results suggested a potential link between TIM-3 expression and lack of treatment response.

<table>
<thead>
<tr>
<th>Tumor location</th>
<th>N of samples (tumor grade)</th>
<th>% of positive samples</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>GEP-NECs: 18 colon-rectum; 6 biliary tract; 3 pancreas; 3 stomach; 1 small bowel; 1 duodenum</td>
<td>37 (37 G3)</td>
<td>14%</td>
<td>(39)</td>
</tr>
<tr>
<td>GEP- and BP-NETs: 21 GEP; 6 lung; 8 genitourinary; 4 head and neck; 7 unknown</td>
<td>57 (3 NET G3; 48 NEC; 6 MINEN)</td>
<td>32%</td>
<td>(40)</td>
</tr>
<tr>
<td>BP-NECs</td>
<td>148 (#LC-NEC)</td>
<td>100%</td>
<td>(41)</td>
</tr>
<tr>
<td>LC-NECs</td>
<td>95</td>
<td>3%</td>
<td>(18)</td>
</tr>
</tbody>
</table>

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<td>LC-NECs</td>
<td>95</td>
<td>3%</td>
<td>(18)</td>
</tr>
</tbody>
</table>

* PD-L1 and PD-1 analyzed in 215 cases; ** PD-L1 analyzed in 98 cases; NP, staining not performed; TILs, tumor infiltrating lymphocytes; TMA, tissue microarray; SI, small intestine; HPF, high power field; BP, bronchopulmonary; LC-NEC, large cell neuroendocrine carcinoma; MINEN, mixed neuroendocrine non-neuroendocrine neoplasm;
Combination IO therapy trials

The Southwest Oncology Group (SWOG) DART (Dual Anti-CTLA-4 and Anti-PD-1 Blockade in Rare Tumors) trial is a phase II basket trial of ipilimumab and nivolumab in rare tumors, including two cohorts of NENs: one defined as “neuroendocrine carcinoma, including carcinoid of the lung” but enrolling well and poorly differentiated NENs of any primary site (48). and the other defined as “endocrine carcinoma of the pancreas and digestive tract”, but enrolling well and poorly differentiated NENs. Patients are eligible if they progressed on at least one prior line of therapy. Study treatment consists of ipilimumab 1 mg/kg every 6 weeks and nivolumab 240 mg every 2 weeks until local investigator determined progression of disease. Preliminary analysis of the “neuroendocrine carcinoma including carcinoid of the lung” cohort was recently presented. Fifty-eight percent of patients had high-grade cancers (differentiation not well defined), 30% had intermediate-grade tumors, and 12% low-grade tumors. The ORR was 24%, all responders with high-grade tumors (which included 2 high-grade lung NECs). Forty-two percent of high-grade tumors responded while none of the low or intermediate grade tumors responded. Six-month PFS was 30% and mean OS was 11 months at the time of data cut-off. The toxicity profile was relatively mild, with 30% of patients reporting fatigue and 27% reporting nausea. Elevated alanine aminotransferase (ALT) was the most common (9%) grade 3-4 immune-related AE.

Towards novel immunotherapeutic strategies

In addition to checkpoint inhibitors, bispecific tumor-targeting antibodies (BsAbs) are a new class of drugs allowing for simultaneous engagement of two targets, theoretically increasing binding specificity, allowing for dual activation or blockade of two disease mediators. A recent, phase I, first-in-human study of a new BsAb (XmAb18087) targeting somatostatin receptor (SSTR) subtype 2 and CD3 in well-differentiated neuroendocrine and gastrointestinal stromal (GIST) tumors began accrual in early 2018 and recruitment is ongoing (NCT03411915).

Adoptive transfer of genetically-modified autologous T cells is gaining traction as one of the most promising advances in cancer immunotherapy, and impressive outcomes have been recently recorded in clinical trials of chimeric antigen receptor (CAR) T cells targeting CD19 or B cell maturation antigen (BCMA) in patients with B cell malignancies (49). CARs are synthetic fusion proteins consisting of an extracellular antigen-recognition domain linked to an intracellular activating domain. Once activated, CAR T cells proliferate and exert their effector functions including lysis of target cells, leading to “epitope spreading” and consequent induction of a secondary immune response against the tumor. Research is currently underway to develop CAR T cells directed against somatostatin receptor-expressing NET cells. Data presented this year show preliminary evidence of antitumor activity against NET cell lines and experiments in mice are currently underway (50).

Oncolytic viruses engineered to selectively kill tumor cells have exhibited activity in melanoma and head and neck cancers (51-53). An oncolytic adenovirus (AdVince) for the treatment of liver metastases from NETs was recently developed and is now being evaluated in a phase I/IIa clinical trial for patients with liver dominant NETs of GEP or bronchial origin (NCT02749331). The adenovirus is designed to utilize the gene promoter from human chromogranin A for selective replication in neuroendocrine cells, and in preclinical evaluation of the virus, was found to successfully replicate in and kill NET cells without inducing a considerable amount of proinflammatory cytokines or chemokines in blood (54).

Conclusions

While the majority of well-differentiated NETs are “immunologically cold,” poorly differentiated NECs are more likely to express PD-L1 in the presence of an abundant T cell infiltration. Single agent PD-1 inhibitor therapy has demonstrated limited activity in well-differentiated NETs, although preliminary evidence suggests that lung NETs may be mildly more immunosensitive than NETs of the GI tract. Surprisingly, PD-1 inhibitors have also shown limited activity in patients with poorly differentiated NECs (excluding Merkel Cell Cancers and Lung NECs, which are biologically distinct). Very early data suggest that combination ipilimumab/nivolumab treatment may be associated with promising activity in poorly differentiated NECs. These preliminary findings require confirmation. Novel immunotherapeutic approaches such as bispecific antibodies and CAR-T cells may one day represent a new paradigm for the treatment of well-differentiated NETs.

Acknowledgments

None.
Footnote

Conflicts of Interest: J Strosberg: Consult (Novartis); Speakers bureau (Ipsen and Lexicon). The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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