Potential new applications of immunotherapy for neuroendocrine neoplasms: immune landscape, current status and future perspectives

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ABSTRACT
Neuroendocrine neoplasms (NENs) are a highly heterogeneous class of tumors arising from neuroendocrine cells and peptidergic neurons. After failure of first-line treatment, patients have poor prognosis and limited treatment options. Immune checkpoint inhibitors (ICIs) may be a powerful means of increasing therapeutic efficacy for such patients, but ICIs alone have low response rates and short disease control durations in most NENs and may be effective for only a portion of the population. ICIs combined with other immunotherapies, targeted therapies, or cytotoxic drugs have achieved some efficacy in patients with NENs and are worthy of further exploration to assess their benefits to the population. In addition, accumulating experimental and clinical evidence supports that the interaction between neuroendocrine and immune systems is essential to maintain homeostasis, and assessment of this broad neuroendocrine-immune correlation is essential for NEN treatment. In this review, we summarize the immune microenvironment characteristics, advances in immunotherapy, predictive biomarkers of ICI efficacy for NENs, and the effects of common endocrine hormones on the immune system, highlighting possible new application areas for this promising treatment in neglected NENs.

KEYWORDS
Neuroendocrine neoplasms; immunotherapy; predictive biomarker; hormones

Introduction

Neuroendocrine neoplasms (NENs) are a highly heterogeneous class of tumors arising from neuroendocrine cells and peptidergic neurons, which express neuroendocrine markers and produce bioactive amines and/or polypeptide hormones. NENs are classified as well-differentiated neuroendocrine tumors (NETs) and aggressive, poorly differentiated neuroendocrine carcinomas (NECs)1,2. NENs may appear in different parts of the body, most commonly in the gastroenteropancreatic compartment (up to 70% of cases) and respiratory tract (approximately 20% of cases)3,5, whereas those arising from other regions, such as the genitourinary tract, the female reproductive system, and Merkel cells of the skin are less common. Their behavior, metastatic potential, and prognosis are highly variable, depending on the site of origin, differentiation grade, and proliferation index4. Treatment approaches for NENs vary widely and are based on the location, grade, and stage of the primary lesion6. The cornerstone treatments for well-differentiated NETs are surgery, local ablation therapy, antisecretory and anti-proliferative drugs, such as somatostatin receptor ligands and peptide receptor radionuclide therapy, and targeted therapies; however, their rates of effectiveness are low7. High-grade NECs are biologically similar to small cell lung cancers (SCLCs), and are characterized by rapid disease progression and high sensitivity to platinum-based chemotherapy, yet no standard regimen exists for NECs after second-line treatment, and patients who fail first-line treatment have very poor prognosis. The reported overall survival (mOS) of patients with poorly differentiated NECs is 11 months, and the progression-free survival (mPFS) is 4 months8,9. Limited drugs are available for advanced NENs, and effective treatments remain lacking.

Immunotherapy is the most important breakthrough in the field of cancer therapy in recent years, and immune checkpoint inhibitors (ICIs) have made major breakthroughs in cancer therapy and have been approved for the treatment of various types of cancer10,11. For NENs, immunotherapy is used...
primarily to treat lung and skin tumors, and ICIs are approved for SCLCs and Merkel cell carcinomas (MCCs)\textsuperscript{12-15}, because both types have high tumor mutational burden (TMB) and environmental causes of immunogenicity\textsuperscript{16}. For other NENs, several early trials and clinical studies have evaluated the efficacy of ICIs and provided preliminary insights into the roles of these therapies. Overall, the results of exploratory studies in NENs have shown that the efficacy of immunotherapy alone is limited but may be considered for portions of the population. ICIs combined with other immunotherapies, targeted therapy, or cytotoxic drugs have achieved some efficacy in patients with NENs and are worthy of further exploration for their benefits to the population. Herein, we summarize the immune microenvironment characteristics, advances in immunotherapy, predictive biomarkers of ICI efficacy for NENs, and the effects of common endocrine hormones on the immune system, highlighting possible new areas of application for this promising treatment in neglected NENs.

**Characteristics of the tumor immune microenvironment of neuroendocrine neoplasms**

NETs usually exhibit an immunologically “cold” tumor immune microenvironment, owing to the lack of immunocompetent cellular components, low tumor antigens, and other factors\textsuperscript{17,18}; in contrast, NECs may be a more suitable target for immunotherapy, given their extensive mutation load and denser immune infiltration\textsuperscript{19}. In terms of PD-L1 expression, 8.99% of G1, 12.37% of G2, 37.04% of G3, and 48.91% of NECs had ≥ 25% positive PD-L1 membrane staining in tumor cells or tumor-infiltrating lymphocytes (TILs)\textsuperscript{20}. Lamarca et al.\textsuperscript{21}, in a similar series of 70 tissue samples from small bowel NETs (sb-NETs), have observed 2% Ki-67 positivity, and PD-L1 positivity (≥ 5% membrane expression) in 12.8% of tumor cells and 24.3% of TILs. PD-L1 expression is significantly associated with a higher WHO tumor grade\textsuperscript{22,23}, and poorer PFS and OS in NENs\textsuperscript{24-26}. A recent analysis of 102 NETs of different grades and primary sites has indicated that PD-L1 expression is highest in pNETs and lowest in ileal NETs, respectively, whereas PD-L2 expression is highest in pancreatic NETs\textsuperscript{27}. In addition, depleted and regulated TILs are enriched in PD-L1-positive NETs but diminished in G3 well-differentiated NETs, thus suggesting that immune tolerance in NETs may be driven by PD-L1/2 expression, and NETs that express PD-L1 and with TILs might benefit from PD-L1 inhibition. However, other studies have found no association between PD-L1 expression and grade or prognosis\textsuperscript{26,28}.

A recently published article analyzing the genomic landscape of late-stage NENs has measured TMB through whole genome sequencing and found a lower TMB for NENs (1.09 mut/Mb) than NECs (5.45 mut/Mb), and a higher number of indels, structural variants, and polyploid genomes in NECs, according to an analysis of the types of genomic alterations\textsuperscript{29}. Similarly, in the well-differentiated pancreatic NET (pNET) cohort reported by Scarpa et al.\textsuperscript{30} (n = 98), the TMB was 0.82 mut/Mb. Moreover, in the grade 3 NET cohort reported by Venizelos et al.\textsuperscript{31} (n = 29), the TMB was 4.6 mut/Mb, and that in the gastro-entero-pancreatic (GEP)-NEC (n = 152) cohort was 5.1 mut/Mb. Notably, lung NETs have been shown to have higher overall TMB levels. A retrospective study by Chi et al.\textsuperscript{32} has reported a TMB of lung NETs of 11.0 mut/Mb, and a retrospective study by Sabari et al.\textsuperscript{33} has found a significantly higher TMB for large cell neuroendocrine carcinomas (LCNECs) than SCLCs (15.3 mut/Mb vs. 8.2 mut/Mb) and non-small cell lung cancers (NSCLCs) (15.3 mut/Mb vs. 5.7 mut/Mb). In addition, higher T cell infiltration in the immune microenvironment has been observed for highly malignant NETs/NECs. Through multiplex fluorescence immunohistochemistry for quantitative analysis, the number of TILs and PD-L1+ TILs has been found to be significantly greater in pNETs than pNETs, and PD-L1 high T-lymphocyte infiltration is significantly greater with increasing grade in pNETs\textsuperscript{17}. Da Silva et al.\textsuperscript{34} have reported T cell immune infiltration, with high density T cell infiltration (CD4+, CD8+, and CD45RO+ cells) in 14%–48% of sb-NETs and 32%–65% of pNETs, and low levels of FOXP3+ regulatory T cells (Tregs) cells in both cohorts. Nevertheless, the immunological characteristics of NENs are not fully understood, and more knowledge regarding the complex immune landscape of these heterogeneous tumors must be obtained to clarify the therapeutic and prognostic value of these NEN characteristics.

**Effects of hormones secreted by the neuroendocrine system on the immune system**

Functional NENs secrete a variety of hormones and cause a variety of neuroendocrine syndromes, thus affecting the tumor immune microenvironment and systemic immune
status. A growing body of experimental and clinical evidence supports that the interaction between the neuroendocrine and immune systems is essential for the maintenance of homeostasis. For example, hormones such as prolactin (PRL), growth hormone, cortisol, and sex hormones regulate the differentiation and function of immune system cells and cytokine production, and vice versa. Assessing this broad neuroendocrine-immune correlation is essential for understanding NENs. Adrenocorticotropic hormone (ACTH) generally suppresses immune responses, but certain functions can be enhanced. For example, in an investigation of the effect of ACTH on cytotoxicity in T lymphocytes previously sensitized in vivo, ACTH showed no significant effect on primary mixed lymphocyte responses but enhanced secondary (memory) cytotoxic responses by as much as 100% after 2 days of treatment. ACTH also inhibits concanavalin A-stimulated T lymphocyte mitosis. Mitotic inhibition is stronger in immature thymocytes than in mature thymocytes. Furthermore, the finding that IFN-γ is elevated in culture suggests that ACTH may enhance memory cytotoxic responses through multiple mechanisms such as direct cellular alterations or synergy with regulatory cytokines. A sexual dimorphism exists in the expression of innate and adaptive immune responses, as a result of the effects of androgens and estrogens on the immune system. Estrogen can promote or protect against autoimmune diseases, and androgens have been described as suppressors of inflammation and immune function, which directly promote neutrophil differentiation from myeloid progenitors, inhibit dendritic cell differentiation and function, and inhibit B-cell and T-cell lymphopoiesis, but may increase the risk of cancer development. Notably, a robust indicator of response to immunotherapy is intratumoral expression of IFNG, which is inhibited by androgens. Inhibition of androgen receptor activity in CD8+ T cells has been found to prevent T cell exhaustion and increase responsiveness to PD-1 targeted therapy by significantly increasing cytokine production and IFN-γ expression in CD8+ T cells. PRL has immunomodulatory effects, and its secretion is stimulated by cytokines such as IL-1 and IL-2, and inhibited by endothelin-3 and IFN-γ. PRL increases IL-2 synthesis and secretion, and stimulates IFN-γ production by natural killer (NK) cells and lymphocytes; promotes maturation of thymic CD4+ T and CD8+ T lymphocytes, and stimulates immunoglobulin production by plasma cells; promotes the development of antigen-presenting cells expressing major histocompatibility class II molecules; and stimulates IL-1β production by macrophages. In addition, a variety of other stimuli also regulate the body’s immunity through diverse mechanisms. For example, growth hormone promotes neutrophil differentiation; antibody and transcription factor synthesis; T cell proliferation, adhesion, and cytotoxic activity; and production of IL-1, IL-2, and IFN-γ.

**Advances in ICIs for neuroendocrine neoplasms**

The immune response process of tumor cells and the main mechanisms of action of ICIs are described in detail in Figure 1. However, evidence of the efficacy and safety of ICIs in the treatment of NENs remains limited; phase I/II trials have evaluated the roles of ICIs and combinations in the treatment of NENs, but randomized controlled phase III trials have not been conducted. A recent systematic review and meta-analysis of 636 patients with NENs treated with ICIs has reported an objective response rate (ORR) of 10%, an overall disease control rate (DCR) of 42%, an mPFS of 4.1 months, and an mOS of 11 months, thereby demonstrating the overall effectiveness of ICIs in the treatment of patients with NENs. Park et al. have systematically evaluated the effectiveness of ICIs in patients with advanced or metastatic NENs. In a pooled analysis of 10 studies with 464 patients, the overall ORR was 15.5%, but the values varied by primary site (thoracic, 24.7%; gastroentero-pancreatic, 9.5%), tumor differentiation (poorly differentiated, 22.7%; well differentiated, 10.4%), and drug regimen (combined therapy, 25.3%; monotherapy, 10.1%). For patient-tailored management, changes in ICI treatment efficacy according to tumor differentiation and drug regimen should be considered. The promising efficacy and favorable safety profiles of ICIs indicate an opportunity to expand the therapeutic promise for NENs. A summary of the main trial results and ongoing studies of immunotherapy and combination therapy is provided below.

**Advances in ICI monotherapy for neuroendocrine neoplasms**

The effectiveness of ICI monotherapy for NENs is limited, particularly for poorly differentiated tumors. Pembrolizumab is the most widely studied immunotherapeutic agent for NENs. The phase Ib Keynote-028 study showed that 16
patients with PD-L1-positive pNECs had an ORR of 6%, and 12-month PFS and OS rates of 27% and 87%, respectively; 25 cohorts of patients with typical carcinoid (TC) or atypical carcinoid (AC) had an ORR of 12%, and 12-month PFS and OS rates of 27% and 65%, respectively. The phase II basket trial Keynote-158 study has reported that, for well-differentiated NETs that failed standard treatment, pembrolizumab treatment had an ORR of only 3.7%, an mPFS of 4.1 months, a 6-month PFS of 39.3%, and an mOS of 24.2 months, with good safety data. Although efficacy is limited in patients with NECs overall, pembrolizumab has been approved by the U.S. Food and Drug Administration (FDA) for the treatment of SCLCs in patients with evidence of disease progression during or after platinum-based chemotherapy and at least one other prior therapy, on the basis of on pooled data from the SCLC cohorts in the Keynote-158 and Keynote-028 trials. In addition, the results of a prospective randomized phase II trial evaluating pembrolizumab in 19 patients with NECs and 9 patients with G3 NETs have shown no response. In a trial of 14 patients with extrapulmonary poorly differentiated NECs, the ORR of pembrolizumab treatment was 7%, and only one patient achieved complete response. In the phase II KEYNOTE-017 trial, the ORR of pembrolizumab in first-line treatment of advanced MCCs was 56% (59% for virus-positive and 53% for virus-negative patients, regardless of PD-L1 expression), the 24-month PFS was 48.3%, the mPFS was 16.8 months, the 24-month OS was 68.7%, mOS was not reached, 28% had grade 3–4 treatment-associated adverse events (TRAES), 7 patients (14%) discontinued pembrolizumab because of AEs, and 1 patient died from treatment. Avelumab is the only PD-L1 inhibitor used as a single agent in prospective clinical trials in GEP-NENs, and 3 phase II clinical trials (NCT03278405, NCT03278379, and NCT03147404) have been conducted to evaluate avelumab.
in patients with G2/3 NETs or NECs. However, no patients achieved an objective response to avelumab treatment. The phase II AVANCE trial assessed the activity of avelumab in 29 patients with high-grade NENs of different origins, with an ORR of 6.9% and mPFS of 16 weeks. In a retrospective study conducted at the Mayo Clinic, 3 patients with G3 NETs showed no objective response to ICI65. In the JAVELIN Merck 200 trial, the ORR of avelumab therapy in a cohort of patients with chemotherapy-refractory MCCs was 33.0%, 74% had a sustained response for more than 1 year, and the treatment was well tolerated; interim results in the first-line MCC cohort with avelumab showed an ORR of 62.1%, an estimated DOR (at least 6 months) of 83%, and no grade 4 or 5 adverse events. Walker et al. confirmed the positive results observed in the JAVELIN trial, in a study of nearly 500 patients with MCCs or progressive RCC. On the basis of these results, the FDA (avelumab and pembrolizumab) and the European Medicines Agency (avelumab) have approved ICIs as first-line or subsequent treatments for MCC. In the ongoing phase I/II CheckMate-358 study (NCT02488759, testing nivolumab treatment in participants with virus-positive and virus-negative solid tumors), the preliminary data for 25 patients with MCCs have indicated an ORR of 68%. The 3-month PFS and OS rates were 82% and 92%, respectively; 20% of patients had grade 3-4 TRAEs, and 12% discontinued treatment because of toxicity.

Spartalizumab is a novel high-affinity humanized anti-PD-1 antibody that blocks the binding of PD-L1/PD-L2 to PD-1. In a phase II trial evaluated spartalizumab in 4 cohorts: well-differentiated (WD) GI-NETs (n = 32), WD pNETs (n = 33), WD thoracic NETs (n = 30), and GEP-NECs (n = 21), with ORRs of 0%, 3%, 20%, and 4.8%, respectively. Interestingly, patients with higher PD-L1 expression or more CD8+ cell infiltration at baseline evaluation showed higher ORR. In a phase II, single-arm, open-label, multicenter study (NCT02955069) exploring the antitumor activity of spartalizumab in previously treated WD NETs of intestinal, pancreatic, and thoracic origin and poorly differentiated GEP-NECs, 5 of the thoracic cohorts (6 TC and 24 AC) achieved a best response of PR; 52% had a sustained response for more than 1 year, and the treatment was well tolerated; interim results in the first-line MCC cohort with avelumab showed an ORR of 62.1%, an estimated DOR (at least 6 months) of 83%, and no grade 4 or 5 adverse events. Walker et al. confirmed the positive results observed in the JAVELIN trial, in a study of nearly 500 patients with MCCs or progressive RCC. On the basis of these results, the FDA (avelumab and pembrolizumab) and the European Medicines Agency (avelumab) have approved ICIs as first-line or subsequent treatments for MCC. In the ongoing phase I/II CheckMate-358 study (NCT02488759, testing nivolumab treatment in participants with virus-positive and virus-negative solid tumors), the preliminary data for 25 patients with MCCs have indicated an ORR of 68%. The 3-month PFS and OS rates were 82% and 92%, respectively; 20% of patients had grade 3-4 TRAEs, and 12% discontinued treatment because of toxicity.

Advances in ICI-based combination regimens for neuroendocrine neoplasms

ICIs combined with chemotherapy

ICI combination chemotherapy has shown unsatisfactory results in NECs. A phase II two-arm clinical trial has investigated pembrolizumab and combination chemotherapy regimens (including irinotecan or paclitaxel) in patients with NECs with poor extrapulmonary differentiation who failed first-line chemotherapy (excluding MCC), but the ORR of the combination group was only 9%, and the mPFS and mOS were 2 and 4 months, respectively, thus suggesting that the efficacy of immune combination chemotherapy in poorly differentiated NECs is limited; however, the study sample size was small. The phase II NICE-NEC trial was the first trial to assess the efficacy of nivolumab in combination with platinum-based doublet chemotherapy as a first-line treatment for GEP or unknown origin grade 3 NENs. Most patients had NECs (68.4%), the Ki67 positivity was > 55% (65.8%), the ORR for the combination treatment was 53%, the mPFS was 5.7 months, and the 12-month OS rate required further follow-up. In the interim analysis of trial NCT03728361, 12 patients (7 with GEP-NETs and 5 with pulmonary carcinoma) were treated with nivolumab combined with temozolomide, with a PR of 25%, SD of 67%, and PD of 8%; however, the follow-up time was short, and follow-up data are expected. On the basis of the phase III IMPower133 trial and the CASPIAN trial, the FDA approved ICI in combination with carboplatin and etoposide as a first-line therapy for patients with extensive-stage SCLCs. However, the phase III trial of ipilimumab in combination with chemotherapy for first-line treatment of extensive-stage-SCLCs indicated no evidence of prolonged OS, and the adverse events were comparable to those observed in the chemotherapy plus placebo group. The CASPIAN phase III trial also assessed the efficacy of...
durvalumab plus platinum plus etoposide or in combination with tremelimumab, or chemotherapy alone. The results of the double immunization combined chemotherapy group are not yet available, the mDOR was 5.1 months, the ORR was 68% and 58%, and the mOS was 13.0 and 10.3 months ($P = 0.0047$), respectively, for the immunization plus chemotherapy group and the chemotherapy alone group, and no significant difference in safety data was observed. In another phase II trial, paclitaxel in combination with pembrolizumab showed moderate activity as a second-line treatment after platinum-etoposide chemotherapy for SCLC, with an ORR of 23.1%, mDOR of 9.1 months, mPFS of 5.0 months, and mOS of 9.1 months. The most common grade 3–4 AEs were febrile neutropenia (7.7%), asthenia (7.7%), hyponatremia (7.7%), and type I diabetes (7.7%). The efficacy of ICIs in combination with other methods to enhance the immune response in SCLCs is currently being evaluated in multiple clinical trials, including ICIs combined with the agonistic monoclonal antibody utomilumab targeting CD137, INCAGN01876 targeting GITR, or INCAGN01949 targeting the CD134 costimulatory receptor; an antibody-drug conjugate targeting DDL3 (Rova-T); an inhibitor targeting multiple kinases, such as VEGF receptor, fibroblast growth factor receptor, and platelet-derived growth factor receptor; anlotinib; and the tubulin polymerization inhibitor plinabulin. Ongoing clinical trials of ICIs in combination with chemotherapy in NECs are detailed in Table 1.

ICIs in combination with anti-angiogenic therapy

A series of preclinical and clinical studies have shown that anti-angiogenic therapy and ICI therapy have mutually enhancing effects. On the one hand, anti-angiogenesis blocks negative immune signaling by increasing the ratio of anti/pro-tumor immune cells and decreasing multiple immune checkpoint expression. On the other hand, ICI treatment can restore the immune support microenvironment and promote vascular normalization. In addition, because vascular normalization enhances drug delivery benefits, lower doses of ICI may be applied, thereby decreasing the risk of adverse events. Halperin et al. have reported that atezolizumab-based bevacizumab resulted in an ORR of 15%–20% and a PFS of 14.9–19.6 months for extrapancreatic and pancreatic grade G1 and G2 NETs, thus indicating synergistic activity of bevacizumab in converting immune “cold” tumors into “hot” tumors. In a phase I clinical study of toripalimab in combination with surufatinib, a small molecule tyrosine kinase inhibitor of VEGFR/fibroblast growth factor receptor/CSF-1R, in advanced solid tumors, the ORR was 44%, and the DCR was 87.5%. Preliminary data from a phase II clinical study (NCT04169672) in a multicenter polyoma cohort have shown that 20 patients with evaluable NECs who received surufatinib in combination with toripalimab had an ORR and DCR of 20% and 70%, respectively, and an mPFS of 3.9 months; 33.3% experienced ≥ grade 3 TRAEs, and 28.6% and 19% discontinued the trial drug, surufatinib or toripalimab, respectively, because of TRAEs. Data on well-differentiated NETs have not been reported.

Several studies of anti-angiogenic agents in combination with immunotherapy are ongoing, and the specific trial contents are detailed in Table 1.

Combination therapy with dual ICIs

Treatments with dual checkpoint inhibitors using anti-PD-1/PD-L1 and anti-CTLA-4 antibodies have shown promising efficacy. The phase II CA209-538 clinical trial of an N plus I regimen in the treatment of NENs (NCT02923934) has demonstrated an overall ORR of 24%, DCR of 72%, and mPFS and mOS of 4.8 months and 14.8 months, respectively; the response rate of bronchial AC was 33%; the ORR was 43% and 33.3% in 7 patients with pNENs and 3 patients with GI-NENs, respectively; and all responders had high-grade disease. A phase II study (NCT04969887) evaluating N plus I in patients with immunotherapy-sensitive cancers (including NECs and G3 NETs) in CA209-538 is registered and is expected to be completed in October 2024. The phase II basket SWOG DART S1609 trial of N plus I in rare tumors (NCT02834013) included 33 patients with low-, intermediate-, and high-grade NETs and NECs, and has shown an ORR of 25% [1 complete response (44%) and 0 responses for high- and low-intermediate NENs, respectively], 6-month PFS of 31% (44% and 14% for high- and low-intermediate NENs, respectively), and mOS of 11 months, thus suggesting that high-grade NENs or NECs may benefit more from N plus I treatment. In terms of safety, 38% of patients experienced grade 3–4 irAEs, and 31.5% of patients discontinued treatment because of irAEs; therefore, immune doublet therapy requires attention to the management of irAEs. Nonetheless, the SWOG DART S1609 trial was cited as class 2B evidence by the 2020 NCCN guidelines for neuroendocrine tumors, which recommend N plus I for the treatment of extrapulmonary non-pancreatic poorly differentiated NECs progressing after chemotherapy. Similarly, 3 included studies evaluated the
| Clinical trials       | Phase | N    | Line                  | Population                                                                                     | Regimen                                                                 |
|----------------------|-------|------|-----------------------|------------------------------------------------------------------------------------------------|
| NCT03008209          | II    | 38   | First-line            | Metastatic SCLCs or poorly differentiated NECs                                                 | Nivolumab + etoposide + carboplatin                                       |
| NCT03901378          | II    | 189  | First-line or second-line | Metastatic or unresectable recurrent LCNECs or GEP-NECs                                       | Pembrolizumab + platinum + etoposide                                      |
| NCT04085651          | II/III| 185  | First-line or second-line | Extrapulmonary poorly differentiated, small cell NECs.                                          | Atezolizumab + cisplatin/carboplatin + etoposide                         |
| NCT0391731           | II    | 144  | ≥Second-line          | Advanced and progressive neoplasms of the endocrine system                                    | Cabozantinib + atezolizumab                                              |
| NCT0400474           | II    | 30   | ≥Second-line          | Advanced solid tumors including low/moderate thoracogenic NETs                                 | Surufatinib + tislelizumab                                               |
| NCT04579757          | Ib/II | 30   | First-line or second-line | Advanced/metastatic, well-differentiated grade 3 NETs or NECs of the pancreas, gastrointestinal tract, lung and unknown primary site. | Nivolumab + 177Lu-DOTATATE                                              |
| NCT03554812 (JAVELIN Medley) | Ib/II | 398  | First-line or second-line | Locally advanced or metastatic solid tumors including advanced/metastatic SCLCs              | Utomilumab + avelumab                                                    |
| NCT03126110          | I/II  | 145  | ≥Second-line          | Advanced or metastatic malignancies including NETs                                              | INCAGN01876 + nivolumab + ipilimumab                                     |
| NCT03241173          | I/II  | 52   | ≥Second-line          | Advanced or metastatic malignancies including NETs                                              | INCAGN01949 + nivolumab, ipilimumab, or both                            |
| NCT03026166          | I/II  | 42   | ≥Second-line          | SCLCs                                                                                          | Rova-T + nivolumab or nivolumab + ipilimumab                             |
| NCT0452682           | II    | 40   | ≥Second-line          | SCLCs                                                                                          | Anlotinib + sintilimab                                                   |
| NCT03575793          | I/II  | 55   | ≥Second-line          | Recurrent SCLCs                                                                                 | Plinabulin + nivolumab + ipilimumab                                     |
| NCT03071406          | II    | 50   | ≥Second-line          | Metastatic MCCs                                                                                | Nivolumab + ipilimumab ± SBRT                                            |
| NCT03304639          | II    | 100  | First-line or second-line | Metastatic MCCs                                                                                | Nivolumab + pembrolizumab or radiation therapy                           |
| NCT04261855          | I/II  | 65   | First-line            | Metastatic MCCs                                                                                | Avelumab + external beam radiation therapy or 177-Lu-DOTATATE             |
| NCT02643303          | I/II  | 58   | Any number of prior systemic therapies | Metastatic MCCs                                                                               | Tremelimumab + durvalumab + polyICLC                                    |

NETs, neuroendocrine tumors. NECs, neuroendocrine carcinomas. NENs, neuroendocrine neoplasms. WD, well differentiated. GEP, gastro-entero-pancreatic. SCLCs, small cell lung cancers. MCC, Merkel cell carcinoma. LCNEC, large cell neuroendocrine carcinoma. N, number of patients expected or actually enrolled.
efficacy of N plus I and consistently observed high ORRs of 24.1%–27.3%\textsuperscript{50,65,80}. However, in the phase III Checkmate-451 trial, N plus I as a first-line maintenance therapy for SCLCs did not show an improvement in survival, and the incidence of all grade adverse effects was higher in the double immunization group (86%) than in the single agent treatment\textsuperscript{81}. In addition, the prospective phase II DUNE trial explored the efficacy of ipilimumab in combination with durvalumab for NENs that failed standard therapy, including 4 cohorts: lung AC/TC (n = 27), G1 and G2 GI-NETs (n = 31), G1 and G2 pancreatic NETs (n = 32), and G3 GEP-NENs (n = 33, including 91% NECs)\textsuperscript{82}. The DCR rate at 9 months was 7.4%, 32.3%, and 25% in the first 3 cohorts, and the OS rate at 9 months was 36.1% in the G3 GEP-NEN cohort; the irORR according to irRECIST criteria was 7.4%, 0%, 6.3%, and 9.1%, and the mPFS was 5.3 months, 8.0 months, 8.1 months, and 2.5 months in the 4 cohorts, respectively. The main grade 3 or higher AEs were hepatotoxicity (9.7%) and diarrhea (6.5%). Therefore, the combination of dual ICIs has limited efficacy and a relatively lower ORR for well-differentiated NETs, whereas it may be more worthy of further investigation for poorly differentiated NECs.

**Other combination immune therapies**

In addition to the above regimens, relevant prospective clinical trials of ICIs in combination with other therapies are currently being conducted in NET cohorts, such as spartalizumab in combination with LAG525 (NCT03365791)\textsuperscript{83}, 177Lu-DOTA0-Tyr3-octreotate (Lu-177) in combination with nivolumab (NCT03325816)\textsuperscript{84}, and pembrolizumab plus somatostatin receptor ligands (NCT03043664). Radiotherapy or peptide receptor radionuclide therapy before the initiation of ICI treatment and induction of inflammation at the tumor level, accompanied by an increase in TILs, may be another approach for exploration. IDO mediated immunosuppression is most prominent in patients with low tryptophan levels; therefore, these patients may be interesting candidates for ICI combined with IDO inhibitor therapy\textsuperscript{85}. Owing to the abundance of TAMs, and their negative correlation with T cell infiltration in the TME of NETs, the combination of CD47 inhibitors with ICIs may also be an interesting option in future studies\textsuperscript{86}. A phase II study (NCT02465957) is testing the advantage of activated NK-92 NK cell infusions in combination with ALT-803 (interleukin-15) in patients with advanced MCC. ALT-803 (NCT03228667) has also been administered in combination with a PD-1/PD-L1 inhibitor for as many as 16 cycles in patients with advanced solid tumors, including MCCs or SCLCs, that progressed after an initial response to PD-1/ PD-L1 inhibition. Interferon-alpha (IFN-a) has been identified as a potential treatment modality for patients with NETs, and patients with metastatic or unresectable NETs including low proliferation rates are currently being recruited for a study evaluating whether this treatment regimen decreases the rate of circulating Tregs with a combination of cyclophosphamide and IFN-a (NCT02838342). In the future, IFN-a therapy in combination with ICIs should be investigated as a combination therapy. Survivin long peptide vaccine, an immune tumor vaccine against NETs, and dendritic cells loaded with autologous tumor homogenates have entered phase I and II clinical trials, respectively. In addition, epigenetic therapy and immunotherapy can be combined to effectively overcome cancer treatment conundrums\textsuperscript{87} and are worthy of exploration in NETs.

**Exploration of predictive biomarkers of ICI efficacy for neuroendocrine neoplasms**

Because the benefits of immunotherapy are usually limited to a subset of patients, the research community has made great efforts to find predictive markers that can identify such patients\textsuperscript{88}. A study in tumors that typically receive immunotherapy has identified biomarkers that may predict response to immunotherapy, including PD-L1 expression, TMB, neoantigen burden, and TILs. Evidence suggests that PD-L1 expression is associated with higher response rates and prolonged survival after anti-PD-1/PD-L1 therapy\textsuperscript{89-91}. In a phase Ib trial of patients with NENs (Ki-67 ≥ 10%) treated with toripalimab, patients with PD-L1 expression ≥ 10% had a better ORR than patients with PD-L1 expression < 10% (50.0% vs. 10.7%, \textit{P} = 0.019)\textsuperscript{92}. However, the ORR in patients with pNETs with positive PD-L1 expression in the Keynote-28 study was low, at 6.3%\textsuperscript{50}; all 4 patients with GEP-NETs who achieved PR in the Keynote-158 study had negative PD-L1 expression\textsuperscript{51}; no differences in DCR, PFS, or OS were observed between the PD-L1-negative and PD-L1-positive arms with G3 NENs in the combined analysis of the 2 prospective, non-randomized trials\textsuperscript{16}. In fact, PD-L1-negative tumors also respond well to anti-PD-1/PD-L1 therapy\textsuperscript{93}. Therefore, PD-L1 must be combined with other predictive biomarkers to better predict the populations that may benefit from immunotherapy. Large
clinical and genomic data sets have shown that high TMB is associated with prolonged survival in patients treated with ICI for various cancer types\textsuperscript{94}. Although pembrolizumab has been approved by the FDA for patients with TMB $\geq 10$ mut/Mb, on the basis of the results of the Keynote-158 trial, regardless of the primary tumor, significant differences exist in TMB among tumor types, and the optimal TMB threshold for each histology is controversial\textsuperscript{95}. In addition, high TMB may be associated with a higher proportion of immunogenic cancer-specific “neoantigen” burden, but these “neoantigen” proteins must be effectively presented and expressed\textsuperscript{96}. In May 2017, the FDA approved pembrolizumab for patients with unresectable or metastatic microsatellite instability (MSI)-high or mismatch repair-deficient (dMMR) solid tumors progressing after prior therapy\textsuperscript{97,98}. However, analysis of 2 studies including 89 patients with small intestinal NETs and 35 patients with pNETs has suggested that in NETs, DNA dMMR is rare, and tumors have microsatellite instability\textsuperscript{99,100}. A study investigating NECs ($n = 53$) and mixed adenoneuroendocrine carcinomas ($n = 36$) has indicated that 12.4% of patients with these carcinomas had MSI\textsuperscript{101}.

Immune cell infiltration in the TME is one of the most essential features for generating an appropriate antitumor immune response. An observational study of 87 patients with NETs has found that in primary moderate NETs, intensive CD3$^+$ T cell infiltration was associated with a relapse-free survival of 128 months, whereas patients with low intratumoral T cell levels had a recurrence free survival of only 61 months\textsuperscript{102}. In the same study, an analysis of 39 patients with NETs with liver metastases showed that the degree of infiltration of CD3$^+$, CD4$^+$, and CD8$^+$ did not predict OS, whereas low levels of infiltrating Tregs predicted prolonged OS\textsuperscript{102}. In addition, chronic inflammation can overstimulate neuroendocrine cells, thus leading to hyperplasia and neoplastic transformation. The neutrophil-lymphocyte ratio and platelet-lymphocyte ratios are simple and effective biomarkers available for patients with advanced cancer including NENs, and their prognostic roles have been confirmed in 15 and 4 studies, respectively; however, the thresholds for both ratios remain undefined\textsuperscript{103}. Finally, the specific composition of the gut microbiome has been shown to influence antitumor immune responses, but no data are available on the gut microbiomes of patients with NETs and NECs treated with ICIs. An in-depth study of the immune microenvironment and the exploration of novel markers are crucial tasks, but the predictive efficiency of molecular markers confirmed by current studies remains unsatisfactory, and the study sample sizes have been small. More predictive immunotherapeutic markers must be explored to identify so-called “hot” tumor lesions and guide immunotherapy.

**Summary and future prospects**

NENs are a rare, complex and highly heterogeneous class of tumors with poor prognosis and limited treatment options for patients after failure of first-line therapy. Immunotherapy may be a powerful means of improving treatment efficacy in such patients, but the optimal strategy remains to be determined. The response rate to ICI monotherapy is low, the disease control time is short, and treatment may be effective for only a portion of the population. The low TMB and often “cold” immune microenvironment suggest that combination therapy may be used to overcome the intrinsic resistance of NENs to immunotherapy, including immune combination chemotherapy or somatostatin analogues and anti-angiogenic drugs, double ICI combinations, or simultaneous combination of anti-angiogenic drugs, to improve patient outcomes. In addition, accumulating experimental and clinical evidence supports that the interaction between neuroendocrine and immune systems is essential to maintaining homeostasis, and assessment of this broad neuroendocrine-immune correlation is essential for NENs. Future efforts should focus on finding the best way to incorporate immunotherapy into NEN treatment, including defining the most appropriate treatment context, combination, and treatment sequence. In addition, accurate molecular typing and immune monitoring are the only way to find markers for combined prediction of therapeutic effects and adverse reactions. However, the overall predictive efficiency of known biomarkers, such as high TMB, PD-L1 and MSI high/dMMR, is poor at present. Gene mutation types, T cell regulation-associated factors, and pathways of NENs involved in immunotherapy must be identified, and a combination of multidimensional, stereoscopic, and dynamic markers must be used to improve the predictive efficacy of markers; reveal the molecular mechanism of PD-1 antibody therapy and the causes of drug resistance; and guide the clinical practice of NEN immunotherapy in the future.

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No potential conflicts of interest are disclosed.

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References

1. Kulke MH, Shah MH, Benson AB, 3rd, Bergsland E, Berlin JD, References Wrote the paper: Rilan Bai.

2. Yao JC, Hassan M, Phan A, Dagogoy C, Leary C, Mares JE, et al. One hundred years after “carcinoid”: epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. J Clin Oncol. 2008; 26: 3063-72.

3. Dasari A, Shen C, Halperin D, Zhao B, Zhou S, Xu Y, et al. Trends in the incidence, prevalence, and survival outcomes in patients with neuroendocrine tumors in the United States. JAMA Oncol. 2017; 3: 1335-42.

4. Lloyd RV, Osamura RY, Kloppe G, Rosai J. Who classification of tumours of endocrine organs. 4th ed. Geneva S: WHO. 2017.

5. Brighi N, Lambert G, Manuzzi L, Maggio I, Campana D. Therapeutic options in lung neuroendocrine tumors: between established concepts and new hopes. Anticancer Drugs. 2019; 30: e00784.

6. Kaderli RM, Spanjol M, Kollár A, Bútikofer L, Gloy V, Dumont RA, et al. Therapeutic options for neuroendocrine tumors: a systematic review and network meta-analysis. JAMA Oncol. 2019; 5: 480-9.

7. Pavel M, O’toole D, Costa F, Capdevila J, Gross D, Kianmanesh R, et al. ENETS consensus guidelines update for the management of distant metastatic disease of intestinal, pancreatic, bronchial neuroendocrine neoplasms (NEN) and NEN of unknown primary site. Neuroendocrinology. 2016; 103: 172-85.

8. Sorbye H, Welin S, Langer SW, Vestermark LW, Holt N, Osterlund P, et al. Predictive and prognostic factors for treatment and survival in 305 patients with advanced gastrointestinal neuroendocrine carcinoma (WHO G3): the NORDIC NEC study. Ann Oncol. 2013; 24: 152-60.

9. Garcia-Carbonero R, Sorbye H, Baudin E, Raymond E, Wiedenmann B, Niederle B, et al. ENETS consensus guidelines for high-grade gastroenteropancreatic neuroendocrine tumors and neuroendocrine carcinomas. Neuroendocrinology. 2016; 103: 186-94.

10. Vaddepally RK, Kharel P, Pandey R, Garje R, Chandra AB. Review of indications of FDA-approved immune checkpoint inhibitors per NCCN guidelines with the level of evidence. Cancers (Basel). 2020; 12: 738.

11. Yoo C, Oh CR, Kim ST, Bae WK, Choi HJ, Oh DY, et al. Systemic treatment of advanced gastroenteropancreatic neuroendocrine neoplasms in Korea: literature review and expert opinion. Cancer Res Treat. 2021; 53: 291-300.

12. Hellmann M, Ott PA, Zugazagoitia J, Ready NE, Spigel DR. Nivolumab (nivo) ± ipilimumab (ipi) in advanced small-cell lung cancer (SCLC): first report of a randomized expansion cohort from CheckMate 032, J Clin Oncol. 2017; 35(suppl 15): e803.

13. Chung HC, Lopez-Martín JA, Kao SC-H, Miller WH, Ros W, Gao B, et al. Phase 2 study of pembrolizumab in advanced small-cell lung cancer (SCLC): KEYNOTE-158. J Clin Oncol. 2018; 36: 8506.

14. Nghiern P, Bhatia S, Daud A, Friedlander P, Klinger H, Kohrt H, et al. PD-L1 Activity of PD-1 blockade with pembrolizumab as first systemic therapy in patients with advanced Merkel cell carcinoma. Eur J Cancer 2015; 51: S20-1.

15. Horn L, Mansfield AS, Szczęsna A, Havel L, Krazkowski M, Hochmair MJ, et al. First-line atezolizumab plus chemotherapy in extensive-stage small-cell lung cancer. N Engl J Med. 2018; 379: 2220-9.

16. Vijayvergia N, Dasari A, Deng M, Litwin S, Al-Toubah T, Alpough RK, et al. Pembrolizumab monotherapy in patients with previously treated metastatic high-grade neuroendocrine neoplasms: joint analysis of two prospective, non-randomised trials. Br J Cancer. 2020; 122: 1309-14.

17. Takahashi D, Kojima M, Suzuki T, Sugimoto M, Kobayashi S, Takahashi S, et al. Profiling the tumour immune microenvironment in pancreatic neuroendocrine neoplasms with multispectral imaging indicates distinct subpopulation characteristics concordant with WHO 2017 classification. Sci Rep. 2018; 8: 13166.

18. De Hosson LD, Takkenkamp TJ, Kats-Ugurlu G, Bouma G, Bulthuis M, De Vries EGE, et al. Neuroendocrine tumours and their microenvironment. Cancer Immunol Immunother. 2020; 69: 1449-59.

19. Cives M, Pelle E, Quaresmini D, Rizzo FM, Tucci M, Silvestris F. The tumor microenvironment in neuroendocrine tumors: biology and therapeutic implications. Neuroendocrinology. 2019; 109: 83-99.

20. Milione M, Miceli R, Barretta F, Pellegrinelli A, Spaggiari P, Tagliabue G, et al. Microenvironment and tumor inflammatory features improve prognostic prediction in gastro-entero-pancreatic neuroendocrine neoplasms. J Pathol Clin Res. 2019; 5: 217-26.

21. Lamarca A, Nonaka D, Breitwieser W, Ashton G, Barriuso J, Milione M, Miceli R, Barretta F, Pellegrinelli A, Spaggiari P, et al. Pembrolizumab ± nivolumab in advanced gastroenteropancreatic neuroendocrine neoplasms: a phase 2, open-label, single-arm, multicentre study. Lancet Oncol. 2020; 21(1): 71-82.

22. Sampredo-Núñez M, Serrano-Somavilla A, Adriados M, Cameselle-Teijeiro JM, Blanco-Carrera C, Cabezus-Agricola JM, et al. Analysis of the tumour microenvironment in neuroendocrine gastroenteropancreatic tumors. Eur J Cancer. 2019; 107: 133-41.
of expression of the PD-1/PD-L1 immune checkpoint system and its prognostic impact in gastroenteropancreatic neuroendocrine tumors. Sci Rep. 2018; 8: 17812.

23. Cavalcanti E, Armentano R, Valentini AM, Chieppa M, Caruso ML. Role of PD-L1 expression as a biomarker for GEP neuroendocrine neoplasm grading. Cell Death Dis. 2017; 8: e3004.

24. Oktay E, Yalcin GD, Ekmecki S, Kahraman DS, Yalcin A, Degirmenci M, et al. Programmed cell death ligand-1 expression in gastroenteropancreatic neuroendocrine tumors. J Buon. 2019; 24: 779-90.

25. Yang MW, Fu XL, Jiang YS, Chen XJ, Tao Y, Yang YJ, et al. Clinical significance of programmed death 1/programmed death ligand 1 pathway in gastric neuroendocrine carcinomas. World J Gastroenterol. 2019; 25: 1684-96.

26. Bösch F, Brüwer K, Altendorf-Hofmann A, Auernhammer CJ, Spitzweg C, Westphalen CB, et al. Immune checkpoint markers in gastroenteropancreatic neuroendocrine neoplasia. Endocr Relat Cancer. 2019; 26: 293-301.

27. Pinato DJ, Vallipuram A, Evans JS, Wong C, Zhang H, Brown M, et al. Programmed cell death ligand expression drives immune tolerogenesis across the diverse subtypes of neuroendocrine tumours. Neuroendocrinology. 2021; 111: 465-74.

28. Ali AS, Langer SW, Federspiel B, Hjortland GO, Janson ET. PD-L1 expression in gastroenteropancreatic neuroendocrine neoplasms grade 3. PLoS One. 2020; 15: e0243900.

29. Van Riet J, Van De Werken HJG, Cuppen E, Eskens F, Tesslera M, Van Veenendaal LM, et al. The genomic landscape of 85 advanced neuroendocrine neoplasms reveals subtype-heterogeneity and potential therapeutic targets. Nat Commun. 2021; 12: 4612.

30. Scarpa A, Chang DK, Nones K, Corbo V, Patch AM, Bailey P, et al. Whole-genome landscape of pancreatic neuroendocrine tumours. Nature. 2017; 543: 65-71.

31. Venizelos A, Elvehakken H, Perren A, Nikolaienko O, Deng W, Lothe IMB, et al. The molecular characteristics of high-grade gastroenteropancreatic neuroendocrine neoplasms. Endocr Relat Cancer. 2021; 29: 1-14.

32. Chi Y, Liu W, Zuo L, Wang Y, Zhao H. Abstract 4743: Genetic characteristics of PanNETs, Rectal NETs, Thoracic NETs and its correlation with efficacy of chemotherapy. Proceedings: AACR Annual Meeting 2020; April 27-28, 2020 and June 22-24, 2020; Philadelphia, PA, 2020.

33. Sabari JK, Julian RA, Ni A, Halpenny D, Hellmann MD, Drilon AE, et al. Outcomes of advanced pulmonary large cell neuroendocrine carcinoma stratified by RB1 loss, SLFN11 expression, and tumor mutational burden. J Clin Oncol. 2018; 36(suppl 15): e20568-e68.

34. Da Silva A, Bowden M, Zhang S, Masugi Y, Thorner AR, Herbert ZT, et al. Characterization of the neuroendocrine tumor immune microenvironment. Pancreas. 2018; 47: 1123-9.

35. Webster JJ, Tonelli L, Sternberg EM. Neuroendocrine regulation of immunity. Annu Rev Immunol. 2002; 20: 125-63.

36. Klein JR. Dynamic interactions between the immune system and the neuroendocrine system in health and disease. Front Endocrinol (Lausanne). 2021; 12: 655982.

37. Johnson EW, Hughes TK, Jr., Smith EM. ACTH enhancement of T-lymphocyte cytotoxic responses. Cell Mol Neurobiol. 2005; 25: 743-57.

38. Wira CR, Rodriguez-Garcia M, Patel MV. The role of sex hormones in immune protection of the female reproductive tract. Nat Rev Immunol. 2015; 15: 217-30.

39. Walker SE. Estrogen and autoimmune disease. Clin Rev Allergy Immunol. 2011; 40: 60-5.

40. Gubbels Bupp MR, Potluri T, Fink AL, Klein SL. The confluence of sex hormones and aging on immunity. Front Immunol. 2018; 9: 1269.

41. Kissick HT, Sanda MG, Dunn LK, Pellegrini KL, On ST, Noel JK, et al. Androgens alter T-cell immunity by inhibiting T-helper 1 differentiation. Proc Natl Acad Sci U S A. 2014; 111: 9887-92.

42. Gubbels Bupp MR, Jorgensen TN. Androgen-induced immunosuppression. Front Immunol. 2018; 9: 794.

43. Ayers M, Lunecford J, Nebozyn M, Murphy E, Loboda A, Kaufman DR, et al. IFN-γ-related mRNA profile predicts clinical response to PD-1 blockade. J Clin Invest. 2017; 127: 2930-40.

44. Prat A, Navarro A, Paré L, Regault N, Galván P, Pascual T, et al. Immune-related gene expression profiling after PD-1 blockade in non-small cell lung carcinoma, head and neck squamous cell carcinoma, and melanoma. Cancer Res. 2017; 77: 3540-50.

45. Bental W, Lieberherr M, Giese G, Wrehlke C, Stamm O, Sekeris CE, et al. Functional testosterone receptors in plasma membranes of T cells. FASEB J. 1999; 13: 123-33.

46. Guan X, Polesso F, Wang C, Sehrawat A, Hawkins RM, Murray SE, et al. Androgen receptor activity in T cells limits checkpoint blockade efficacy. Nature. 2022; 606: 791-6.

47. Bernard V, Young J, Binart N. Prolactin - a pleiotropic factor in health and disease. Nat Rev Endocrinol. 2019; 15: 356-65.

48. Borba VV, Zaandman-Goddard G, Shoenfeld Y. Prolactin - a pleiotropic factor in non-small cell lung carcinoma, head and neck squamous cell carcinoma, and melanoma. Sci Rep. 2018; 8: 17812.

49. Weigent DA. Lymphocyte GH-axis hormones in immunity. Cell Immunol. 2011; 40: 60-5.

50. Mehnert JM, Bergsland E, O’neil BH, Santoro A, Schellens JHM, Cohen RB, et al. Pembrolizumab for the treatment of programmed death-ligand 1-positive advanced carcinoid or pancreatic neuroendocrine tumors: results from the KEYNOTE-028 study. Cancer. 2020; 126: 3021-30.

51. Strosberg J, Mizuno N, Doi T, Grande E, Delord JP, Shapira-Frommer R, et al. Efficacy and safety of pembrolizumab in previously treated advanced neuroendocrine tumors: results from the phase II KEYNOTE-158 study. Clin Cancer Res. 2020; 26: 2124-30.

52. Yao JC, Strosberg J, Fazio N, Pavlov ME, Pavel ME, Bergsland E, Ruszniewski P, et al. Spartalizumab in metastatic, well/poorly-differentiated neuroendocrine tumors. Endocr Relat Cancer. 2021; 28: 161-72.

53. Patel SP, Othus M, Chae YK, Giles FJ, Hansel DE, Singh PP, et al. A phase II basket trial of dual Anti-CTLA-4 and Anti-PD-1 blockade in rare tumors (DART SWOG 1609) in patients with nonpancreatic neuroendocrine tumors. Clin Cancer Res. 2020; 26: 2290-6.
54. Lu M, Zhang P, Zhang Y, Li Z, Gong J, Li J, et al. Efficacy, safety, and biomarkers of toripalimab in patients with recurrent or metastatic neuroendocrine neoplasms: a multiple-center phase Ib trial. Clin Cancer Res. 2020; 26: 2337-45.

55. Riesco Martínez MC, Capdevilla J, Alonso V, Jimenez-Fonseca P, Teulé A, Grande E, et al. Nivolumab plus platinum doublet chemotherapy as first-line therapy in unresectable, locally advanced or metastatic G3 neuroendocrine neoplasms (NEs) of the gastroenteropancreatic (GEP) tract or unknown (UK) origin: preliminary results from the phase II NICE-NEC trial. Ann Oncol. 2021; 32(suppl 5): S908-9.

56. Bongiovanni A, Maiorano BA, Azzali I, Liverani C, Bocchini M, Fausti V, et al. Activity and safety of immune checkpoint inhibitors in neuroendocrine neoplasms: a systematic review and meta-analysis. Pharmaceuticals (Basel). 2021; 14: 476.

57. Park EJ, Park HJ, Kim KW, Suh CH, Yoo C, Chae YK, et al. Efficacy of immune checkpoint inhibitors against advanced or metastatic neuroendocrine neoplasms: a systematic review and meta-analysis. Cancers (Basel). 2022; 14: 794.

58. Chan JA, Raj NP, Aggarwal RR, Calabrese S, Demore A, Dhawan MS, et al. Phase II study of pembrolizumab-based therapy in previously treated extrapulmonary poorly differentiated neuroendocrine carcinomas: results of Part B (pembrolizumab + chemotherapy). J Clin Oncol. 2021; 39(suppl 15): 4148-48.

59. Fang L, Arvind D, Dowlati A, Mohamed A. Role of immunotherapy in gastro-enteropancreatic neuroendocrine neoplasms (GEP-NENs): current advances and future directions. J Neuroendocr. 2021; 33: E12943.

60. Ott PA, Bang YJ, Piha-Paul SA, Razak ARA, Bennouna J, Soria JC, et al. T-cell-inflamed gene-expression profile, programmed death ligand 1 expression, and tumor mutational burden predict efficacy in patients treated with pembrolizumab across 20 cancers: KEYNOTE-028. J Clin Oncol. 2019; 37: 318-27.

61. Nghiem P, Bhatia S, Lipson EJ, Sharman WH, Kudchadkar RR, Brohl AS, et al. Durable tumor regression and overall survival in patients with advanced Merkel cell carcinoma receiving pembrolizumab as first-line therapy. J Clin Oncol. 2019; 37: 693-702.

62. Kaufman HL, Russell J, Hamid O, Bhatia S, Terheyden P, D’angelo SP, et al. Avelumab in patients with chemotherapy-refractory metastatic Merkel cell carcinoma: a multicentre, single-group, open-label, phase 2 trial. Lancet Oncol. 2016; 17: 1374-85.

63. Abstracts presented at the 13th Annual multidisciplinary neuroendocrine tumor medical virtual symposium of the North American Neuroendocrine Tumor Society, October 2-3, 2020. Pancreas. 2021; 50: 441-67.

64. Fottner CAL, Ferrata M, Krug S, Michl P, Schad A, Roth W, et al. A Phase II, open label, multicenter trial of Avelumab in patients with advanced, metastatic high-grade neuroendocrine carcinomas NEC G3 (WHO 2010) progressive after first-line chemotherapy (AVENEC). J Clin Oncol. 2019; 37: 4103.

65. Gile JJ, Liu AJ, McGarrah PW, Eiring RA, Hobday TJ, Starr JS, et al. Efficacy of checkpoint inhibitors in neuroendocrine neoplasms: mayo clinic experience. Pancreas. 2021; 50: 500-5.

66. Kelly K, Infante JR, Taylor MH, Patel MR, Wong DJ, Iannotti N, et al. Safety profile of avelumab in patients with advanced solid tumors: a pooled analysis of data from the phase 1 JAVELIN solid tumor and phase 2 JAVELIN Merkel 200 clinical trials. Cancer. 2018; 124: 2010-7.

67. D’angelo SP, Russell J, Lebbé C, Chmielowski B, Gambichler T, Grob JJ, et al. Efficacy and safety of first-line avelumab treatment in patients with stage IV metastatic Merkel cell carcinoma: a preplanned interim analysis of a clinical trial. JAMA Oncol. 2018; 4: e180077.

68. Walker JW, Lebbé C, Grignani G, Nathan P, Dirix L, Fenig E, et al. Efficacy and safety of avelumab treatment in patients with metastatic Merkel cell carcinoma: experience from a global expanded access program. J Immunother Cancer. 2020; 8: e003513.

69. Topalian SL, Bhatia S, Hallebeekc A, Awada A, Boer PJD, Kudchadkar RR, et al. Abstract Ct074: Non-comparative, openlabel, multiple cohort, phase 1/2 study to evaluate nivolumab (NIVO) in patients with virus-associated tumors (Checkmate 358): efficacy and safety in Merkel cell carcinoma (MCC). Cancer Res. 2017; 77(13 suppl): Ct074.

70. Yao JC, Strosberg J, Fazio N, Pavel ME, Ruzniewski P, Bergland E, et al. Activity & safety of spartalizumab (PDR001) in patients (Pts), with advanced Neuroendocrine Tumors (NET) of Pancreatic (Pan) Gastrointestinal (GI), or Thoracic (T) Origin, & Gastroenteropancreatic Neuroendocrine Carcinoma (GEP NEC) who have progressed on prior treatment (Tx). Ann Oncol. 2018; 29(suppl 8): viii467-78.

71. Owen DH, Wei L, Goyal A, Zhou S-A, Jacob R, et al. CLO20-054: a phase 2 trial of nivolumab and temozolomide in advanced neuroendocrine tumors (NETs): interim efficacy analysis. J Natl Compr Canc Netw. 2020; 18. DOI:10.6004/jnccn.2019.7460.

72. Gandhi L, Rodriguez-Abreu D, Gadgeel S, Esteban E, Felip E, De Angelis F, et al. Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer. N Engl J Med. 2018; 378: 2078-92.

73. Beck M, Luft A, Szczesna A, Havel L, Kim SW, Akerley W, et al. Pembrolizumab in patients with refractory small-cell lung cancer. Lung Cancer. 2019; 136: 122-8.

74. Yi M, Jiao D, Qin S, Chu Q, Wu K, Li A. Synergistic effect of leaves of five mulberry accessions and cataloguing structural
and expression variants for future prospects. PLoS One. 2021; 16: e0252246.

79. Shen L, Yu X, Lu M, Zhang X, Su W. Surufatinib in combination with toripalimab in patients with advanced neuroendocrine carcinoma: results from a multicenter, open-label, single-arm, phase II trial. J Clin Oncol. 2021; 39(suppl 15): e16199-e99.

80. Klein O, Kee D, Markman B, Michael M, Underhill C, Carlino MS, et al. Immunotherapy of ipilimumab and nivolumab in patients with advanced neuroendocrine tumors: a subgroup analysis of the CA209-538 clinical trial for rare cancers. Clin Cancer Res. 2020; 26: 4454-9.

81. Owonikoko TK, Kim H, Govindan R, Ready N, Reck M, Peters S, et al. Pembrolizumab (Nivo) plus ipilimumab (Ipi), nivo, or placebo (Pbo) as maintenance therapy in patients (Pts) with extensive disease small cell lung cancer (ED-SCLC) after first-line (1L) platinum-based chemotherapy (Chemo): results from the double-blind, randomized phase III checkmate 451 study. Ann Oncol. 2020; 31: ii77.

82. Capdevila J, Teule A, López C, García-Carbonero R, Benavent M, Custodio A, et al. 1157O - A multi-cohort phase II study of durvalumab plus tremelimumab for the treatment of patients (pts) with advanced neuroendocrine tumors of the lung. J Immunother Cancer. 2020; 8: e000980.

83. Uboha NV, Milhem MM, Kovacs C, Amin A, Magley A, Purkayastha DD, et al. Phase II study of spartalizumab (PDR001) and LAG525 in advanced solid tumors and hematologic malignancies. JCO. 2019; 37(suppl 15): 2533.

84. Kim C, Liu SV, Subramanian DS, Torres T, Loda M, Esposito G, et al. Phase I study of the 177Lu-DOTA2-Tyr3-Octreotate (lutatera) in combination with nivolumab in patients with neuroendocrine tumors of the lung. J Immunother Cancer. 2020; 8: e000980.

85. Long GV, Dummer R, Hamid O, Gajewski T, Caglevic C, Dalle S, et al. Epacadostat (E) plus pembrolizumab (P) versus pembrolizumab alone in patients (pts) with unresectable or metastatic melanoma: Results of the phase 3 ECHO-301/KEYNOTE-252 study. J Clin Oncol. 2018; 36(suppl 15): 108.

86. Advani R, Flinn I, Popplewell L, Forero A, Bartlett NL, Ghosh N, et al. CD47 blockade by Hu5F9-G4 and rituximab in non-hodgkin's lymphoma. N Engl J Med. 2018; 379: 1711-21.

87. Zhang N, Hao X. Epigenetic modulation of the tumor immune microenvironment by nanoinducers to potentiate cancer immunotherapy. Cancer Biol Med. 2021; 19: 1-3.

88. Kiyotani K, Toyoshima Y, Nakamura Y. Personalized immunotherapy in cancer precision medicine. Cancer Biol Med. 2021; 18: 955-65.

89. Schachter J, Ribas A, Long GV, Arance A, Grob JJ, Mortier L, et al. Pembrolizumab versus ipilimumab for advanced melanoma: final overall survival results of a multicentre, randomised, open-label phase 3 study (KEYNOTE-006). Lancet. 2017; 390: 1853-62.

90. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N Engl J Med. 2015; 373: 1627-39.

91. Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Catósi T, Fülöp A, et al. Pembrolizumab versus Chemotherapy for PD-L1-positive non-small-cell lung cancer. N Engl J Med. 2016; 375: 1823-33.

92. Shah MH, Goldner WS, Benson AB, Bergsland E, Blaszkowsky LS, Brock P, et al. Neuroendocrine and adrenal tumors, Version 2.2021, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2021; 19: 839-68.

93. Wang VE, Urisman A, Abalacker L, Ali S, Miller V, Aggarwal R, et al. Checkpoint inhibitor is active against large cell neuroendocrine carcinoma with high tumor mutation burden. J Immunother Cancer. 2017; 5: 75.

94. Samstein RM, Lee CH, Shoushtari AN, Hellmann MD, Shen R, Janjigian YY, et al. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. Nat Genet. 2019; 51: 202-6.

95. Marabelle A, Fakih M, Lopez J, Shah M, Shapira-Frommer R, Nakagawa K, et al. Association of tumour mutational burden with outcomes in patients with advanced solid tumours treated with pembrolizumab: prospective biomarker analysis of the multicohort, open-label, phase 2 KEYNOTE-158 study. Lancet Oncol. 2020; 21: 1353-65.

96. Necchi A, Raggi D, Gallina A, Ross JS, Fare E, Giannatempo P, et al. Impact of molecular subtyping and immune infiltration on pathological response and outcome following neoadjuvant pembrolizumab in muscle-invasive bladder cancer. Eur Urol. 2020; 77: 701-10.

97. Masuda K, Banno K, Yanokura M, Kobayashi Y, Kisu I, Ueki A, et al. Relationship between DNA mismatch repair deficiency and endometrial cancer. Mol Biol Int. 2011; 2011: 256063.

98. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med. 2015; 372: 2509-20.

99. Kidd M, Eick G, Shapiro MD, Camp RL, Mane SM, Modlin IM. Microsatellite instability and gene mutations in transforming growth factor-beta type II receptor are absent in small bowel carcinoid tumors. Cancer. 2005; 103: 229-36.

100. Arnason T, Sapp HL, Rayson D, Barnes PJ, Drewniak M, Nassar BA, et al. Loss of expression of DNA mismatch repair proteins is rare in pancreatic and small intestinal neuroendocrine tumors. Arch Pathol Lab Med. 2011; 135: 1539-44.

101. Sahnnane N, Furlan D, Monti M, Romualdi C, Vanoli A, Vicari E, et al. Microsatellite unstable gastrointestinal neuroendocrine carcinomas: a new clinicopathologic entity. Endocr Relat Cancer. 2015; 22: 35-45.

102. Katz SC, Donkor C, Glasgow K, Pillarisetty VG, Gonen M, Espat NJ, et al. T cell infiltrate and outcome following resection of intermediate-grade primary neuroendocrine tumours and liver metastases. HPB (Oxford). 2010; 12: 674-83.

103. Giannatempo P, Necchi A, La Salvia A, Rizza L, Muscogiuri G, Campione S, Pozza Sahnane N, Furlan D, Monti M, Romualdi C, Vanoli A, Vicari E, et al. Microsatellite unstable gastrointestinal neuroendocrine carcinomas: a new clinicopathologic entity. Endocr Relat Cancer. 2021; 19: 839-68.