Immunotherapy in Gastroenteropancreatic Neuroendocrine Neoplasia

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Keywords

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Abstract

The worldwide prevalence and incidence of gastroenteropancreatic neuroendocrine neoplasms (GEP-NENs) and of NENs, in general, have been increasing recently. While valuing the considerable progress made in the treatment strategies for GEP-NEN in recent years, patients with advanced, metastasized disease still have a poor prognosis, which calls for urgent novel therapies. The immune system plays a dual role: both host-protecting and "tumor-promoting." Hence, immunotherapy is potentially a powerful weapon to help NEN patients. However, although recent successes with checkpoint inhibitors have shown that enhancing antitumor immunity can be effective, the dynamic nature of the immunosuppressive tumor microenvironment presents significant hurdles to the broader application of these therapies. Studies led to their approval in NEN of the lung and Merkel cell carcinoma, whereas results in other settings have not been so encouraging. Oncolytic viruses can selectively infect and destroy cancer cells, acting as an in situ cancer vaccine. Moreover, they can remodel the tumor microenvironment

toward a T cell-inflamed phenotype. Oncolytic virotherapy has been proposed as an ablative and immunostimulatory treatment strategy for solid tumors that are resistant to checkpoint inhibitors alone. Future efforts should focus on finding the best way to include immunotherapy in the GEP-NEN treatment scenario. In this context, this study aims at providing a comprehensive generalized review of the immune checkpoint blockade and the oncolytic virotherapy use in GEP-NENs that might improve GEP-NEN treatment strategies.

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Introduction

Over the past decade, cancer immunotherapy has emerged as a novel and critical approach in oncology. Leveraging the body's immune system to thwart cancer is a rapidly developing strategy. Several therapeutic classes have emerged within cancer immunotherapy. Among these, the immune checkpoint inhibitors (ICIs) have had remarkable success across multiple malignancies. However, the cancer immunotherapy pipeline also includes other types of agents. Oncolytic viruses (OVs) are one such immunotherapeutic tools, feverously evaluated in oncology with over 30 different types of OVs being ex-

explored either as a single agent or in combination with other antitumor agents, including ICIs [1].

Neuroendocrine neoplasms (NENs) are rare tumors, but their incidence has increased in the last few years and is estimated to be around 7 cases per 100,000 [2]. NENs arise from the diffuse neuroendocrine cell system and may occur at various disease sites. Most frequently, these neoplasms occur in the gastroenteropancreatic (GEP) system (around 70% of cases), followed by the lung (around 20% of cases) [2, 3]. The World Health Organization (WHO) 2010 classification divides GEP-NENs into G1, G2, and G3, according to Ki-67 and/or mitotic index (MI) [4]. In 2017, WHO published a new classification which divides pancreatic NENs (pan-NENs) against several criteria: proliferation parameters (Ki-67 and/or MI), morphological features (well-differentiated [WED] vs. poorly differentiated [POD] tumors), and the percentage of neuroendocrine component [3]. Thanks to this classification, WED-NENs can be further divided into neuroendocrine tumors (NETs) G1 (Ki-67 < 3%; MI < 2/10 high-power fields [HPF]), NET G2 (Ki-67 3–20%; MI 2–20/10 HPF), and NET G3 (Ki-67 > 20%; MI > 20/10 HPF). On the other hand, POD tumors are classified as neuroendocrine carcinomas (NECs) G3, having by definition Ki-67 > 20%. High-grade NENs include not only POD-NECs, but also a subset of WED-NETs. This recent recognition, formally introduced in the revised 2017 WHO classification of pan-NENs [3] was extended in 2019 to all GEP-NENs [5]. Little data exist to help define the morphological criteria this subdivision should be based on. However, organoid growth pattern, capillary network in direct contact to tumor cells and absence of desmoplastic stroma were found the best to separate NET G3 from NEC and showed prognostic significance [6]. Whether the NEC group should be further subdivided according to proliferation rate remains to be decided, though [7]. However, further studies [8, 9] of the European Neuroendocrine Tumor Society (ENETS) 2017 Guidelines on POD-GEP neoplasms [10] revealed that a 55% Ki-67 cutoff can discriminate POD-NENs with different median overall survival (OS) and response to therapy. Indeed, a higher median OS in patients with Ki-67 ≤ 55% [11] or NET G3 [6] is associated with a lower responsiveness to platinum or platinum/etoposide-based therapy [11], which is conversely highly efficacious in POD-NENs with Ki-67 > 55% [6, 11].

GEP-NENs are also the most prevalent NENs at advanced disease stages, making their management extremely challenging [12]. Another key limitation in the efficient management of GEP-NENs is their high intratumoral heterogeneity, affecting the treatment response and impairing an accurate stratification of the patient co-

hort. Survival is poor in patients with GEP-NENs, ranging from 38 months in patients with localized disease to 5 months in the metastatic setting [13]. Despite unquestionable steps forward, the current therapies for nonresectable GEP-NENs are rather limited and differ depending on the WED-NETs and NECs subtype, the location of the primary lesion and tumor grading. For advanced unresectable WED-GEP-NENs, mainstays of treatment are somatostatin analog (SSA), tyrosine kinase inhibitors (TKIs)-sunitinib, mammalian target of rapamycin (mTOR) inhibitors-everolimus and peptide receptor radionuclide therapy (PRRT), while the platinum based chemotherapy is the first-line treatment for GEP-NECs [1, 14]. However, chemotherapy does not provide a sustained response over time [7]. In most of the cases, the other therapies cannot efficiently control the disease either. A lot of patients do not respond to the chosen agent or develop resistance. Other drawbacks would be their high toxicity (something that cannot be neglected) or their poor availability (PRRT, e.g., is unfortunately available only in specialized centers). These explain the increased interest in finding new therapies for GEP-NENs.

Immunotherapy dramatically changes the natural history of many cancers, and clinical trials are ongoing in different NENs. IFN- α was first introduced in the clinic to treat midgut carcinoids in the 1980s and had encouraging results [15]. Using the immune checkpoint blockade and oncolytic virotherapy in several types of cancers has yielded impressive results, including POD-NENs such as small-cell lung cancer (SCLC), Merkel cell carcinoma, or melanoma [16–19].

Our work aimed to offer an overview of the current knowledge on immunotherapy in GEP-NENs. We focus on GEP-NENs, where there is a significant unmet clinical and therapeutic need, and minimal progress in patient management has been made in nearly 4 decades. Therefore, this study summarizes the results of clinical trials conducted so far in the immune check point blockade field and the preclinical data of the OV's use in GEP-NENs.

Methods

We performed a literature search by MEDLINE (PubMed database) and Google Scholar and we also considered the trials registered on clinicaltrials.gov to identify potentially relevant articles on immunotherapy with ICIs and/or OVs in GEP-NENs. The search was last updated on February 28, 2021. In our literature review, the search strategy included the following terms “immunotherapy” OR “immune checkpoint inhibitor” OR “immune checkpoint blockade” OR “OV” OR “oncolytic virotherapy” OR “oncolytic immunotherapy” OR “oncolytic vaccine” AND “GEP-NENs”

OR “GEP-NETs” OR “GEP-NECs” OR “pancreatic-NENs/NETs” OR “small intestinal (SI)-NENs” OR “midgut carcinoid.” Only articles available in full text for review and published in English were considered. Additional studies were identified by reviewing the references of all selected articles.

Immune Checkpoint Inhibitors as Anticancer Agents

The balance between stimulating and inhibitory signals regulating the action and proliferation of immune cells directs the immune response. The interaction of several proteins located on the membrane of T-cells and antigen-presenting cells (APCs), referred to as immune checkpoints modulates the antitumor immune response [20]. Tumors can escape the immune system recognition through expression ligands that interact with immune checkpoints expressed on T-cells, such as cytotoxic T lymphocyte-associated protein-4 (CTLA-4) and programmed cell death 1 (PD-1) [21, 22]. In recent years, different types of ICIs, consisting of monoclonal antibodies targeting CTLA-4 or PD-1 on T-cells or programmed cell death ligand 1 (PD-L1) on tumor cells were successfully tested in several tumors. The interruption of the immunological shutdown being sent to T cells proved to significantly change the clinical practice and improve patients' outcomes [1, 16, 23, 24].

Immune Checkpoint Inhibitors and GEP-NENs

The therapeutic scenario for NENs changed with the addition of immunotherapy. Immune checkpoint inhibition proved effective in some difficult-to-treat NENs, such as SCLC and Merkel cell carcinoma [16, 19, 24, 25]. Immune cells including B and T cells, natural killer cells, mast cells, tumor-associated macrophages (TAMs), and dendritic cells have been reported to infiltrate GEP-NENs. Additionally, the cytokine and chemokine milieu including tumor necrosis factor- α (TNF- α), IL-2, vascular endothelial growth factor (VEGF)-A, C-X-C motif chemokine receptor 4 and interferon (IFN), as well as immunomodulatory factors including CD73, CD133, CD166, and CD56 have also been identified in GEP-NENs [26]. These observations justified the hypothesis that GEP-NENs might benefit from the use of immunotherapy [27]. Additional biologic rationale for evaluating ICIs in G3 NENs include their high rate of PD-L1 expression (ranging from 14% to 50% of tumors) and relatively high mutational load, which is thought to increase the

chances of immune recognition of neoantigens, compared to low-intermediate-grade NETs [28–30].

However, GEP-NET and NEC reported only low response rates to anti-PD1 monotherapies so far [31–33] and the benefit of immunotherapy is usually limited to a subset of patients. Tumor immune microenvironment (TME) is considered a key in the success of immunotherapy. Recently, several studies have tried to characterize the TME of GEP-NENs, in the need to find reliable predictive biomarkers that could guide clinical decisions. The efficacy of anti PD-1/PD-L1 treatment seems partly dependent on PD-L1 expression [34–36] and T-cell infiltration [37]. Moreover, the antigenicity of the tumor seems to also play a role. An important factor that determines the antigenicity is the amount of neoepitopes, which is mainly determined by the tumor mutational burden (TMB), but also human leukocyte antigen heterozygosity [38–42].

However, the immunoprofiling of TME in GEP-NET/NEC revealed no signs of an activation of the adaptive immune system like PD-L1 expression on immune cells or intratumoral infiltration of T cells expressing T-cell exhaustion markers like PD-1 or T-cell immunoglobulin and mucin domain-containing protein 3. The expression of IFN inducible genes was also low. Instead, it was reported an increased mRNA expression of chemokines, which attract myeloid cells in NEN and a high abundance of genes with immunosuppressive functions like CD47 and CD74 [43]. Interestingly, another recent study demonstrated differential immune-related gene expression across the previously defined 4 transcriptomic pan-NET subtypes: metastasis-like primary (MLP)-1, MLP-2, insulinoma-like, and intermediate. The MLP-1 subtype was identified as showing overexpression of PD-L1, PD-L2, and other markers of sensitivity to immunotherapy, including the T-cell gene expression profile (GEP) signature, as compared to the other 3 pan-NET molecular subtypes. The T cell-inflamed GEP is based on 18 genes co-expressed with IFN- γ and has been reported to predict response to PD-1 blockade in a number of different solid tumors [28]. Though further investigation of this interplay is needed, it seems that selected pan-NET (MLP-1) patients are more likely to respond to ICI therapy [44].

The expression of PD-L1 and PD-1 appears quite heterogeneous across different studies [27]. Some reports argue that PD-L1 is expressed in 9.56–30% of all GEP-NENs [29, 45–47] and 97% of pan-NENs, while there is no expression in SI-NENs [48, 49]. Moreover, in GEP-NENs, but not in SI-NENs subtype, if considering exclusively, PD-L1 expression has been associated with higher

grade GEP-NENs (i.e., NET G3 and NECs), lymphatic metastasis, and poor prognosis [50, 51] and decreased progression-free survival and OS in metastatic patients [29, 47, 52, 53]. The extent of tumor infiltration by immune cells appears to be higher in pan-NETs than midgut NETs and higher in NECs than well-differentiated tumors [54]. In SI-NENs, duodenal NENs display higher immune infiltration than jejunal or ileal NENs [55]. Moreover, exhausted and regulatory tumor-infiltrating T lymphocytes (TILs) were enriched in PD-L1-positive GEP-NECs but decreased in G3 WED-NETs and were significantly associated with shorter patient survival [49, 50]. On this basis, GEP-NECs are regarded as having a “hot” TME with more significant TIL infiltration than GEP-NETs. Overall, it seems that G3 (GEP-NETs and NECs) are the best candidates for treatment with anti-PD-1 antibodies. In line with this, activation of adaptive immunity (co-expression of CD3, CD4, CD8, PD-1, and PD-L1) was demonstrated at least in a subset of G3 POD (GEP-NECs) [56]. On the contrary, well-differentiated GEP-NETs appear suboptimal candidates for immunotherapy, at least theoretically. This difference might be also explained, at least partially, by the fact that NETs are mismatch repair proficient [54] and TMB-high and microsatellite instability (MSI)-high cases have a lower prevalence in low-grade GEP-NENs than in high-grade neoplasms [57].

Interestingly, a deeper characterization of TME has also led to the identification of additional TAMs that have been shown to play a central role in promoting angiogenesis and suppressing the T-cell-mediated antitumoral activity [14]. Increased TAM infiltration is also associated with higher grade GEP-NENs [58]. Furthermore, with increasing pan-NEN grade, the number of PD-1 high T cells and PD-1 high M2 TAMs was enhanced, which may be a predictor of poor survival [26]. Additionally, the expression of CD73 by tumor cells is emerging as a predictive biomarker of immunotherapy response, due to its immunoinhibitory function that protects tumor cells from the immune attack [59]. In gastrointestinal (GI)-NENs, CD73 expression has shown to characterize about 27.2% of neoplasms, besides being associated with increased malignancy and higher PD-L1 expression [45]. In pan-NENs, on the other hand, increased CD73 expression was associated with aggressive tumor behavior and adverse prognostic factor [60]. Moreover, these findings suggest that targeting CD73 in GEP-NENs may enhance the efficiency of anti-PD-1 monotherapy [14].

Though these studies identified critical TME components with clinical significance in GEP-NENs, there is

much more to know about the immune landscape of GEP-NENs. This explains the need for more refined and reliable predictive biomarkers or for a biomarker-based score to predict the sensitivity to ICIs, for personalized immunotherapy treatments. PD-L1 protein expression on tumor cells is currently the best predictive biomarker that immunotherapy benefits from [46, 61, 62]. Besides this higher expression of PD-1/PD-L1, other predictive markers of the response to the immune checkpoint blockade in GEP-NENs include a T-cell-inflamed GEP, lymphocyte infiltration, mismatch repair deficiency, and consequently, TMB and neoantigen load [44, 57, 63]. Few studies support the use of ICIs in GEP-NENs, and the current knowledge is mainly based on a few phase Ib/II studies and some case reports [64–66].

The phase Ib KEYNOTE-028 study evaluated the safety and efficacy of pembrolizumab in pretreated patients, with PD-L1-positive advanced NETs. Sixteen patients with well-differentiated or moderately differentiated pan-NETs and 25 patients with carcinoids received the PD-1 inhibitor as monotherapy, showing an objective response rate (ORR) of 6.3% and 12%, respectively. The OS were 87% and 65%, respectively [67].

The subsequent phase 2 KEYNOTE-158 study [33] investigated pembrolizumab in 107 patients with heavily pretreated, well-differentiated, and progressive NETs (Table 1). The study findings are generally consistent with previous results from the phase 1b KEYNOTE-028 clinical trial. However, in contrast to KEYNOTE-028, patients in the present study were enrolled regardless of tumor PD-L1 expression. After a median follow-up of 24 months, the ORR was only 3.7%, with 3 and 1 partial responses (PRs) recorded in patients with pancreatic and rectal NETs, respectively. It is noteworthy that the ORR in the pan-NETs subgroup was 7.5% (3 out of 40), and all responding patients had PD-L1-negative tumors. As for the role of the TMB in predicting the efficacy of immunotherapy, 2 objective responses (40%) were recorded in the group of 5 patients with TMB-high tumors, whereas only one response out of 82 (1.2%) was reported in the TMB-low group [68].

Sustaining these results, another 2 open-label, phase 2 studies enrolling patients with G3NEN (Ki-67 > 20%) progressing on platinum-based chemotherapy (48% patients had GI primaries while 34% originated in the pancreas) concluded that single-agent Pembrolizumab can be safely administered to patients with G3NENs but has limited activity as a single agent (NCT02939651) [31]. Another finding was that despite a higher baseline PD-L1 expression (47% with positive staining), there was no cor-

Table 1. Clinical trials of check point inhibitors in GEP-neuroendocrine neoplasia

The agent/agents and dosage	Patient population characteristics	ORR (RECIST 1.1)	Trial name/reference
Pemrolizumab 10 mg/kg every 2 weeks	16 patients with PD-L1 – well or moderately differentiated pancreatic NETs 25 patients with PD-L1-positive, locally advanced or metastatic carcinoids (lung, gut, and other locations)	6.3% 12%	Phase Ib KEYNOTE 028 study2 NCT02054806 Mehnert et al. [67]
Pembrolizumab 200 mg every 3 weeks	107 patients with heavily pretreated, well-differentiated, progressive NETs arising in the lung, appendix, small intestine, colon, rectum, or pancreas	3.7% *in the pancreatic NET subgroup: 7.5%	Phase 2 KEYNOTE-158 study NCT02628067 Strosberg et al. [33]
Pembrolizumab 200 mg every 3 weeks	29 patients with advanced G3 NETs/NECs (Ki-67 > 20%) (most patients had GI primaries (48%) while 34% originated in the pancreas) progressing on platinum-based chemotherapy	3.4% (1 patient)	NCT02939651 Vijayvergia et al. [31]
Pembrolizumab (200 mg q3w) + lanreotide depot (90 mg q3w)	22 patients with nonresectable, recurrent or metastatic, well or moderately differentiated GEP-NETs	39% stable disease, 52% progressive disease	NCT03043664 Phase Ib/II Planet study
Spartalizumab 400 mg every 4 weeks	116 patients with advanced thoracic/GEP-NETs and GEP-NECs (enrolled regardless of PD-L1 expression) 32 patients with well-differentiated (G1/G2) GI and 33 with pancreatic NET and a cohort of 21 patients with POD GEP-NEC *The rate of expression of PD-L1 in immune cells: 42% in POD GEP-NECs and 23% in well-differentiated GEP-NETs	3.1%, 3.0%, and 4.8%, respectively	NCT02955069 Yao et al. [32]
Ipilimumab 1 mg/kg every 6 weeks; nivolumab 240 mg every 2 weeks	32 patients of whom 15 with GI-NEN (without pancreatic-NET) and 6 with lung-NEN	Overall: 25% 44% in nonpancreatic high-grade NEC and 0% in low-intermediate grade tumors	DART trial (NCT02834013) Patel et al. [70]
Ipilimumab 1 mg/kg every 3 weeks for 4 doses and nivolumab 3 mg/kg, followed by nivolumab 3 mg/kg every 2 weeks for up to 96 weeks	29 patients with advanced, any grade NENs (including atypical bronchial carcinoid and high-grade pan-NENs)	Overall: 24% 43% of patients with pan-NENs achieved an objective response	NCT02923934 Klein et al.[71]
Bevacizumab 15 mg/kg every 3 weeks and atezolizumab 1,200 mg	NET and NEC (any site), including a pancreatic and an extra-pancreatic NET cohort each enrolling 20 pretreated patients with advanced progressive, grades 1–2 NET	20% in the pancreatic NET cohort (+median PFS of 19.6 months). 15% in the extra pancreatic NET cohort (+median PFS of 14.9 months).	Phase II basket NCT03074513 trial Halperin et al. [72]
Durvalumab (20 mg/kg q4w) + and tremelimumab (1 mg/kg q4w)	123 patients with advanced or metastatic GEP/lung NEN or NEN of unknown primary (excluding lung primaries)	0% in the low-to-intermediate grade GI NETs cohort. 6.3% in the low-to-intermediate grade pancreatic NETs cohort. 9.1% in the high-grade GEP-NENs cohort (<i>by irRECIST criteria</i>).	Phase II study DUNE (NCT03095274) Capdevila et al. [73]
Toripalimab 3 mg/kg via IV infusion once every 2 weeks	40 patients with NENs (Ki-67 ≥ 10%) – of whom 9 with pan-NENs	13.0% (for extra-pancreatic GI derived NENs), 22.2% (for pan-NENs) and 37.5% (for nondigestive NENs)	NCT03167853 Lu et al. [63]
Avelumab 10 mg/kg every 2 weeks	29 patients with advanced G3 NECs (12 pancreatic-NENs and 17 extrapancreatic-NENs)	32% (4 stable disease, 2 partial response) (<i>by irRECIST criteria</i>)	A phase II, open label, multicenter trial (AVENEC) NCT03352934 Fottner et al. [74]

PD-L1, programmed cell death ligand 1; GEP-NEN, gastroenteropancreatic neuroendocrine neoplasm; GI, gastrointestinal; irRECIST, immune-related RECIST; NET, neuroendocrine tumors; NEC, neuroendocrine carcinoma; ORR, objective response rate; pan-NEN, pancreatic NEN; POD, poorly differentiated; PFS, progression-free survival

relation between PD-L1 expression and response to therapy. The 1 patient who responded to treatment in this cohort had a high number of TILs despite negative PD-L1 expression.

Moreover, the phase Ib/II PLANET study investigates the combination of pembrolizumab and lanreotide treatment in nonresectable, recurrent or metastatic, well or moderately differentiated GEP-NETs (NCT03043664). Roughly, only 39% of patients achieved stable disease (SD) [69].

The open-label phase II spartalizumab (a humanized mAb against PD-1) trial also includes a cohort of patients with well-differentiated (G1/G2) GI-NET ($N = 32$) and pan-NET ($N = 33$) and a cohort of patients with POD GEP-NEC ($N = 21$) who have progressed on prior treatment (NCT02955069). ORR was 3.1%, 3.0%, and 4.8%, respectively, in these cohorts, showing a marginal activity in this setting [32].

The dual blockade of PD-1 and CTLA-4 was recently investigated in patients with NEN. In the DART trial (NCT02834013), 15 patients with GI-NET (without pan-NET) received a combination of nivolumab plus ipilimumab. The objective responses were observed only in high-grade NEC (ORR: 44%), with poor efficacy in the well-differentiated forms [70] (Table 1). Though these results require validation in more extensive prospective trials, they suggest that a combination of CTLA-4 and PD-1 inhibition may yield a higher response rate than pembrolizumab monotherapy in nonpan-G3NEN. In another phase 2 trial [27, 71], 29 patients with any grade, advanced NENs received the same combined therapy: ipilimumab and nivolumab (Table 1). At the time of data cutoff, the ORR was 24%, with objective responses observed in 43% of patients with pan-NENs.

Phase II basket NCT03074513 trial tested bevacizumab (a recombinant humanized monoclonal antibody that blocks angiogenesis by inhibiting VEGF-A) and atezolizumab (PD L1 inhibitor) on solid tumors, including NECs and NETs of any site (Table 1). Recently, data from the pancreatic and extra-pancreatic grades 1–2 NET cohorts (extra-panNET) were presented and showed an ORR of 20% and 15%, respectively [72], demonstrating moderate clinical activity in patients with advanced NETs, while the results from the NEC cohort are eagerly awaited.

The multi-cohort, open single-arm phase II study DUNE (NCT03095274) [73] has investigated the efficacy of PD-L1 and CTLA-4 blockade by durvalumab and tremelimumab. The investigation was performed on patients with advanced or metastatic GEP-lung NEN G1/G2

or GEP-NEN G3 or NEN of unknown primary (excluding lung primaries) after progression to previous therapies. Of note, the number of patients with PD-L1-positive tumors was very low, only – 4 patients in the pan – NET cohort. However, after a median follow-up of 10.8 months, the ORR by iRECIST criteria was 0% in the low-to-intermediate grade GI-NETs cohort, 6.3% in the low-to-intermediate grade pan-NETs cohort, and 9.1% in the high-grade GEP- NENs cohort respectively, suggesting limited activity in patients with advanced NETs of GEP origin.

Toripalimab, a humanized IgG4 antibody specific for the human PD-1 receptor, which was first approved to treat second-line metastatic melanoma in China in 2018, was also recently tested in a phase Ib trial that included pretreated NEN patients ($Ki67 > 10\%$). Nine patients presented a pancreatic origin, while, among extra-pancreatic origins, the most common were colorectal, stomach, duodenum, and esophageal primary. The results showed that toripalimab demonstrated antitumor activity and safety in treating recurrent or metastatic NENs (Table 1). Patients with positive PD-L1 expression, TMB-H (top 10%), and/or microsatellite instable (MSI-H) might preferentially benefit from the treatment. The genomic mutation of ARID1A and high genomic rearrangements might be correlated with clinical benefits [63].

Finally, combined data from 2 *phase 2* trials (NET-001 [NCT03278405] and NET-002 trial [NCT03278379]) indicate that avelumab monotherapy had limited antitumor activity in patients with grade 2/3, well-differentiated GEP and lung NENs, with no objective responses achieved in the NCT03278379 trial. As for the AVENEC trial (NCT03352934) (NEC G3 patients, including NET G3, who were progressive after first-line chemotherapy), immune checkpoint blockade with the anti-PD-L1 antibody avelumab in pre-treated high-grade NEN demonstrated relevant activity in a subset of patients with excellent tolerability. The disease control rate after 8 weeks was 32%, with 2 PRs in an interim analysis [65, 74].

To conclude, PD-1 inhibitors such as pembrolizumab and spartalizumab revealed an overall limited antitumoral activity in GEP-NEN patients, highlighting the minimal activity of single-agent immunotherapy. Also, in contrast to SCLC, the activity of pembrolizumab monotherapy in GEP-NENs appears relatively independent of the PD-L1 status [64, 75]. However, the current knowledge of the efficacy of immunotherapy in GEP-NENs is not mature. Overall, even if some evidence suggests the potential for good outcomes with ICIs in high-grade, POD GEP-NENs, immunotherapy in unselected GEP-

Table 2. Preclinical studies of OV use in GEP-neuroendocrine neoplasia

Type of virus/OV	In vitro studies – cell lines	In vivo studies – cell line-derived xenograft mouse models	Construction/genetic modifications	Reference
Adenoviruses (DNA virus) Serotype 5/Ad5fk FWKT (CgA-E1AmiR122)	Human pancreatic NET-BON. Human ileal (midgut) carcinoid – CNDT2.5. Freshly midgut carcinoid cells from liver metastases.		The human CgA promoter controls E1A mRNA expression, leading to preferential E1A expression and hence virus replication in NET cells and cells of neuroendocrine or endocrine origin. Six copies of the mir122 target sequences in the 3'-UTR of the E1A gene leads to hepatocyte-specific degradation of E1A mRNA, and hence blockage of virus replication in normal liver cells, in case of leakiness from the CgA promoter. Cyclic residues from somatostatin (FWKT) were introduced in the fiber knob of the adenovirus.	Leja et al. [98]
Adenoviruses/Tat-PTD-modified Ad5	Human ileal carcinoid – CNDT2.5	CNDT2.5 xenograft mouse model	The PTD of the Tat protein from HIV-1 was introduced in the HVR5 of the hexon protein of Ad5	Yu et al. [99]
Adenoviruses/AdVince (pAd5 PTD [CgA-E1AmiR122])	Human pancreatic NET-BON and QGP-1. Freshly isolated midgut carcinoid cells (from liver metastasis).	Xenograft mouse model using BON cell line	Combining 3 targeting strategies (CgA promoter, miR122 target sequences, and PTD)	Yu et al. [86]
Adenoviruses/AdSur-SYE	Human pancreatic NET: QGP-1, human pancreatic NEC: A99	Xenograft mouse model – QGP1 subcutaneous tumors	Survivin promoter-regulated E1 region and the pancreatic tumor-targeting ligand, SYE, incorporated in the HI loop on the fiber knob of adenovirus	Yamamoto et al. [93]
HSV-1, DNA virus/ Talimogene laherparepvec (T-VEC)	Human pancreatic NET-BON-1 and QGP-1 and human intestinal NEC: HROC-57 and NEC-DUE1		ICP34.5 and ICP47 deletion GM-CSF insertion	Kloker et al. [84]
HSV-1/oHSV T-01 [G47Δ mutant]	Human pancreatic NET:QGP1	Xenograft mouse model- QGP1 subcutaneous tumors	ICP34.5 deletion, ICP6 deletion, ICP47 deletion and LacZ insertion	Matsushima et al. [110]
VACVs (DNA virus)/ GL-ONC1 [GLV-1 h68]	Human pancreatic NETs (BON-1, QGP-1) and intestinal NECs (HROC-57 and NEC-DUE1)		Three expression cassettes encoding β-glucuronidase, β-galactosidase, as well as the Ruc-GFP marker gene – inserted into a VACV backbone derived from the Lister strain	Kloker et al. [113]

GEP, gastroenteropancreatic; CgA, chromogranin A; NEC, neuroendocrine carcinoma; GM-CSF, granulocyte macrophage-colony stimulating factor; OV, oncolytic virus; NET, neuroendocrine tumor; HSV, herpes simplex virus; VACV, vaccinia virus; PTD, protein transduction domain; HIV, human immunodeficiency virus; Tat, transactivator of transcription; HVR, hypervariable region.

NENs seems unfeasible. The immunosuppressive “cold” TME is responsible at least partly for preventing many patients from experiencing benefits.

On the other hand, although ICIs are generally well-tolerated, their use is associated with several adverse effects including the occurrence of severe immune-related adverse events, such as hypophysitis, diabetes, thyroid dysfunctions and neurological issues. Moreover, a consistent portion of patients, in general, either do not respond to the chosen ICI or develop resistance.

Advantages of Using OVs as Anticancer Agents

With the advancement of molecular biology, virology, immunology, and genetic engineering, OVs are increasingly used as an innovative form of cancer immunotherapy. Evidence of their antitumor activity has been already reported in patients with melanoma or head and neck cancer.

These viruses are naturally occurring or can be modified to selectively infect/target tumor cells, to produce enormous amounts of viral progeny within and thus to

damage them harshly, resulting in significant rates of tumor cell lysis, without damaging normal tissues. However, the key mechanism of oncolytic virotherapy is thought to be a secondary antitumor immune response induced by the inflamed lytic TME. OV can recruit and activate tumor-infiltrating immune cells by releasing a large number of tumor antigens from dying tumor cells and secreting cytokines [76–78], which disables immune evasion mechanisms of the tumor [79, 80]. Hence, infections by OV proved to turn immunosuppressive “cold” TMEs into “hot” ones.

An attractive feature of an OV is that it can be combined with other immunotherapy approaches such as ICIs or CAR-T immunotherapy. Moreover the application of OVs for tumor imaging is also under development. OVs carrying reporter genes can replicate and express genes of interest selectively in tumor cells, thus improving in vivo noninvasive precision molecular imaging and radiotherapy [81, 82]. And finally, because of the advancement of virus recombination and genetic modification, as well as the specific mechanisms of oncolytic virotherapy, severe adverse events caused by oncolytic virotherapy have rarely been reported, while milder adverse events can generally be controlled or disappear spontaneously [81]. Multiple viruses are currently under investigation, including herpesvirus, adenovirus, poxvirus, picornavirus, and reovirus as possible oncolytic treatments [83]. But so far, a first OV only was approved for advanced stages of malignant melanomas. Several other OVs are currently in phase III testing [83].

OVs and GEP-NENs

So far, immunotherapies and especially immunovirotherapies are not established as novel treatment modalities for NENs. Actually, only very few, preclinical approaches using oncolytic virotherapy in NEN treatment have been described so far [84–86]. NENs are somewhat neglected in the field of OV therapy although the Seneca Valley virus NTX-010 (or SVV-001), a naturally selected virus for NEN, has been evaluated in clinical phase-I trials both for adults [87] and children [88, 89] with good safety data. When it comes to GEP-NENs, the literature is even more scarce. For GEP-NENs, adenovirus, herpes simplex virus (HSV), and vaccinia virus (VACV) have been investigated. They are, thus, described in more detail below.

Development of Oncolytic Adenoviruses for GEP-NENs

Oncolytic adenoviruses based on human serotype 5 are among the best-studied viruses, owing to their high-titer production and highly effective infection of a wide spectrum of dividing and nondividing cells in vitro and in vivo. Two types of adenoviruses are currently used to restrict the viral replication to cancer cells without affecting normal cells. The first type introduces a mutation in the adenoviral E1A gene. Genetic mutations functionally complement the mutated gene in cancer cells, such as p53 mutations or abnormalities in the retinoblastoma pathway. The second type involves constructing adenoviruses in which the transcription of E1 genes is restricted to cancer cells by tumor or tissue-specific promoters such as the prostate-specific antigen, survivin, chromogranin, midkine, and telomerase reverse transcriptase promoters [90–92].

Virotherapeutic approaches with adenoviruses for NENs are merely in preclinical testing [85, 93]. Therefore, the results, so far, are available only from in vitro studies testing the efficacy of engineered adenoviruses on human endocrine pancreatic tumor cell lines and ileal NET cells (also known as midgut carcinoids) and from in vivo researches on xenografted human NETs. So far, there is only 1 ongoing, clinical phase I/II study using a genetically engineered adenovirus (NCT02749331) in NENs of GEP or bronchial origin, exhibiting liver metastases.

Ad5fk^{FWKT} (CgA-E1AmiR122)

The use of replication-selective oncolytic adenoviruses has not been employed for GEP-NEN until the research work of Leja J., Yu D. & Essand group. They have systematically engineered human adenovirus Ad5 for liver metastases from GEP-NETs therapy using transcriptional targeting and transductional targeting.

Hence, by using the chromogranin A (CgA) promoter to control E1A expression, they created an adenovirus, Ad (CgA-E1A), which selectively replicates in neuroendocrine cells, including the human pancreatic NET cell line BON-1 and neuroblastoma. The same virus was capable to efficiently repress the growth of fast-growing human BON carcinoid tumors in an experimental mouse model when injected intratumorally [94]. Furthermore, they incorporated target sequences for the liver-specific microRNA miR122 in the 3'-UTR of E1A, yielding Ad (CgA-E1AmiR122). Their purpose was to obtain specific degradation of E1A mRNA and efficient blockage of virus replication in normal hepatocytes [95]. This approach decreased liver toxicity significantly.

Ad5 infection is mediated by binding the virus fiber knob to coxsackie-adenovirus receptors (CARs), the na-

tive receptor for adenovirus infection, on target cells. Several studies demonstrated loss of CAR expression on tumor cells from some NETs and thereby intrinsic resistance to treatment with oncolytic Ad5 viruses [96, 97]. However, these tumors often express high levels of somatostatin receptors (SSTRs). Therefore, the team sought to modify OV infectivity and increase virus uptake in SI-NET cells by changing the HI loops of the fiber knob of the adenovirus and introducing the binding motif of octreotide (FWKT) [85]. This led to the increased and selective infection of NET cells, which express high levels SSTR2 [98]. The engineered FWKT-modified virus, Ad-5fk^{FWKT} (CgA-E1AmiR122), showed strong replication and efficient killing of the carcinoid cell line BON. Besides, it bonded to SSTR 2 on NET cells and transduced midgut carcinoid cells from liver metastases about 3–4 times better than nonmodified Ad5. At the same time, it transduced normal hepatocytes at about 50% of Ad5. Modification of fiber and hexon proteins can also remove native immunogenic epitopes and make the virus less visible to the immune system [98].

The same team has also developed an adenovirus having a second, CAR-independent route of cell entry, aiming to enhance transduction of tumor cells with low CAR expression. It was achieved by introducing a cell-penetrating peptide – the protein transduction domain (PTD) of the transactivator of transcription (Tat) protein from human immunodeficiency virus (HIV)-1, in the hypervariable region 5 (HVR5) of the hexon protein, the most abundant protein of the virus capsid. The Tat-PTD-modified Ad5 showed a dramatically increased infection and spread capacity of CAR-negative human carcinoid tumor 2 (CNDT2) cells, compared to unmodified Ad5. More than this, the Tat-PTD-modified virus was significantly better than the nonmodified virus to control NET cell growth and prolong survival in mice in the CNDT2.5 xenograft model [99].

AdVince

In a later study, the team of Essand M. has developed AdVince by combining the 3 previously described targeting strategies (CgA promoter, miR122 target sequences, and PTD), an oncolytic adenovirus designed specifically for the treatment of liver metastases from NET and NEC [86]. The major drawback for evaluating oncolytic human adenovirus is the lack of suitable animal models supporting virus replication. Therefore, the activity and efficacy of clinical-grade AdVince were evaluated on pan-NET cell lines and on freshly SI-NET cells resected from the liver metastases of 3 NET patients. Furthermore, the

in vivo efficacy of AdVince was assessed on a mouse model of pan-NET based on BON cells (Table 2). In addition, the toxicity was investigated on normal hepatocytes from 5 healthy but deceased donors as well as in fresh human blood. AdVince efficiently infected NET cells, replicated therein, and killed the cells without inducing a considerable amount of proinflammatory cytokines or chemokines in blood. Moreover, the combination of using the CgA promoter and miR122-detargeting sequences in controlling E1A expression, together with the Tat-PTD modification led to a 73-fold discrepancy between AdVince activity in SI-NET cells versus normal hepatocytes [86].

These encouraging preclinical studies developed by the team of Essand M. provided the basis for a phase I/IIa clinical trial with AdVince. This ongoing clinical study (NCT02749331) aims to evaluate the safety of repeated infusions of AdVince into the hepatic artery of patients with confirmed progressive NEC of GEP or bronchial origin with multiple liver metastases. They also intend to determine, if possible, the maximum tolerated dose.

AdSur-SYE

In parallel with Essand team's research, Yamamoto et al. [93] constructed a survivin promoter-regulated adenovirus displaying the pancreatic cancer-targeting sequence SYENFSA (SYE) (AdSur-SYE). SYE was selected from the adenovirus library established by screening against the AsPC-1 pancreatic cancer cell line. It was chosen because it was found to significantly enhance the gene transduction efficiency of the adenoviral vector in pancreatic cancer cell lines but not in normal cells [100, 101]. The SYE sequence was further combined with a surviving promoter-regulated oncolytic adenovirus (AdSur-SYE), in which the native tropism was ablated [102]. In human surgical specimens, the AdSur-SYE showed high gene transduction efficiency for pan-NETs and PDAC, 9.1- and 6.2-fold, respectively, compared to that of the nontargeting virus (AdSur). Their results demonstrated that AdSur-SYE exerted strong antitumor efficacy in human pan-NET cell lines and a murine model in which AdSur-SYE effectively proliferated and spread, compared to AdSur. The effect of AdSur-SYE was almost the same as that of AdSur in other cancer and normal cells. The higher oncolytic activity of the SYE-displaying oncolytic adenovirus compared to AdSur is a promising next-generation therapy for advanced, metastatic pan-NEN [93].

Development of Oncolytic HSVs for GEP-NENs

Oncolytic HSV (oHSV) has also been extensively used for a broad spectrum of cancer entities and is under Phase I to III clinical trials to treat solid cancer [103–106]. The oncolytic vector talimogene laherparepvec (T-VEC, HSV type 1), a first-generation HSV-1-based OV with an excellent safety profile in clinical studies, has become the first-ever FDA/EMA-approved OV and is now being used for the treatment of patients with advanced melanoma [107]. T-VEC is a genetically modified HSV type 1. The viral gene responsible for virulence has been deleted to ensure tumor-specific replication (ICP 34.5). Another gene responsible for typically reducing viral immunogenicity (ICP 47) was also deleted. Instead, a GM-CSF transgene has been inserted to enhance the stimulation of the immune system [108]. The first clinical trial for this OV was conducted in 2006 [105] and approved in 2015 as a second-line treatment for late-stage melanoma [109]. Besides melanoma, T-VEC is under clinical investigation for various tumor entities such as liver tumors (NCT02509507), pancreatic cancer (NCT03086642), breast cancer (NCT02658812), or sarcoma (NCT03069378).

But, until the research of Kloker et al. [84], it has not been investigated for its efficacy in NENs. So their study presents a first preclinical assessment of the antitumor potency of this OV against NETs. For this purpose, viral infection, replication, and tumor cell lysis are assessed in a panel of human NET/NEC cell lines derived from lung/pan-NETs and intestinal NECs [84]. Of note, it could be shown that T-VEC infects, replicates in, and lyses human NET/NEC cells exhibiting high oncolytic efficiencies already at relatively low virus concentrations, underscoring the effectiveness of viral replication in NET/NEC cells. Furthermore, when comparing its antiproliferative activity to the one of the mTOR inhibitor Everolimus, no additive effects of combining T-VEC and Everolimus could be detected. Actually, the efficacy of the combination therapy was not superior to the one of the more effective monotherapy. Moreover, Everolimus was not found to alter/influence T-VEC replication. Finally, ganciclovir was shown to limit the replication of T-VEC, thus establishing an important safety feature for future treatments of NEN patients. Although this study constitutes only a first in vitro assessment, including the well-known limitations in clinical predictability, it is expected to lay the foundation for future in vivo studies employing T-VEC in GEP-NENs [84].

In parallel with the study of Kloker et al. [84], Matsuhashima et al. [110] evaluated the in vitro cytopathic effects of a third-generation oHSV T-01 in a panel of cell lines established from human/mouse NET/NEC. These cell lines in-

cluded human pan-NET (QGP1), human pulmonary NET cells (NCI-H727), and murine NET cells (STC-1). Mouse models with subcutaneously implanted human NET QGP1 cells were used to investigate T-01 efficacy in vivo.

In the oHSV T-01, which has a genomic structure similar to that of oHSV G47 Δ mutant [111], the α 47 and γ 34.5 loci are deleted from the HSV-1 genome, and the LacZ gene replaces the ICP6 gene. This gives T-01 a cancer cell-selective replication ability while maintaining the safety profile. Cancer cells are destroyed by the direct cell-killing action of the recombinant HSV-1 (T-01) and the enhanced antitumor efficacy via T cell-mediated immune responses. Hence, T-01 was found to effectively inhibit the growth of human hepatocellular carcinoma and hepatoblastoma in mouse models [112]. It is currently undergoing clinical trials in humans for brain tumors and prostate cancer [110, 111].

Besides, T-01 showed cytotoxicity against the 3 NET/NEC cell lines in vitro and inhibited tumor growth derived from QGP1 cells in a xenograft model. The antitumor effects of T-01 were dependent on the virus concentration and the frequency of administration [110]. Hence, OV therapy using a third-generation HSV-1 may become a new therapeutic approach for NET that can be further combined with anticancer drugs or immune CPIs.

Development of Oncolytic VACV GLV-1h68 for GEP-NENs

After preclinically testing T-VEC in human NEC, Kloker et al. [113] evaluated the potential of GLV-1 h68 to kill cells originating from NEC. GLV-1 h68 (proprietary name GL-ONC1) carries 3 separate transgenic expression cassettes (encoding β -glucuronidase, β -galactosidase, and the Ruc-GFP marker gene) inserted into a VACV backbone derived from the Lister strain. It has demonstrated its safety throughout the years, serving as a major smallpox vaccine. These triple insertions reduce the replication of GLV-1 h68 in healthy cells and favor its replication in tumor cells [114, 115]. They also allow the monitoring of virus activities in cancer patients [116]. In line with the basic characteristics of VACVs, GLV-1 h68 has the advantage of a stable cytoplasmic replication which avoids further virus-driven mutations in cancer cells or healthy cells [117]. Further, as this OV is not targeted to a specific type of tumor, oncolytic activity has already been detected in a broad spectrum of tumor entities in preclinical models as well as in several clinical trials [116, 118–120]. Currently, there are 3 active clinical studies (NCT02759588, NCT02714374, and NCT01766739) that employ GLV-1 h68/GL-ONC1.

Fig. 1. The antitumor effects of combining OV with anti-PD1/PD-L1 therapy. The immune checkpoint receptor PD-1 and its ligands – PD-L1/PD-L2 – play a critical role in the formation of an immunosuppressive TME, which, in its turn, is a crucial regulator of carcinogenesis. OV antitumor activity depends on both direct malignant cell destruction and stimulating systemic antitumor immunity. Briefly, DAMPs, viral protein sequences (PAMPs), and cytokines, released into the TME when an infected cell is lysed, lead to the maturation of APCs. The subsequent activation of APCs then recruits CD4⁺ and CD8⁺ cells to destroy cells expressing viral antigens on tumors. Moreover, the virus-induced inflammatory response can turn immunosuppressive “cold” TMEs into “hot” ones and it can overcome a tumor’s systemic resistance to immune checkpoint blockade. Finally, the enhanced expression of cytokines and chemokines, including TNF- α and types I and II TNFs (especially IFN- γ) induced by virus infection, is likely one of the mechanisms for increased PD-L1 expression in tumor cells but also in immune cells. However, higher PD-L1 in the TME may be

an immune escape mechanism for the virus, since this inhibits functional activity of cytotoxic lymphocytes which will then not attack tumor cells, thus suppressing systemic antitumor immunity. However, this can also sensitize the cells as highly susceptible targets for anti-PD-L1 antibody-mediated therapy. Therefore, in a dual therapy setting (OV combined with check point inhibitors), the PD-L1 antibody can target various types of immune cells, including TAMs, and exhausted CD8⁺ T cells to relieve the immunosuppression functions, but also cancer cells that have survived the OV attack, expressing PD-L1 [133, 137]. This therapeutic approach should elicit more potent and sustained systemic antitumor immunity, with better therapeutic efficacy (created with Bio-Render.com). APC, antigen-presenting cell; PD-1, programmed cell death 1; TAM, tumor-associated macrophage; TNF, tumor necrosis factor; OV, oncolytic virus; TME, tumor microenvironment; DAMPs, danger-associated molecular patterns; PAMPs, pathogen-associated molecular patterns.

In the study of Kloker et al. [113], tumor cell lines originating from pan-NETs (BON-1 and QGP-1), lung NETs (H727 and UMC-11), and intestinal NECs (HROC-57 and NEC-DUE1) were evaluated for their susceptibility to VACV-mediated virotherapy. GLV-1

h68 provided evidence of significant oncolytic effects and exhibited stable cytotoxicity throughout NEN cells from several anatomical origins. Susceptibility to GLV-1 h68 treatment was found to be dose-dependent. Comparing these results to other OVs already tested in NENs,

GLV-1 h68 showed favorable cytotoxicity for pan-NETs and NECs [113]. The oHSV T-VEC was found to be particularly effective in lung and pan-NETs previously. Thus, it requires lower virus concentration (multiplicity of infections [MOIs; i.e., infectious particles per cultured cell]) than GLV-1 h68 for relevant cytotoxicity [84]. AdVince required a MOI of at least 1 to reduce cell viability of primary cells derived from metastatic SI-NETs in the already mentioned preclinical evaluation [86]. Furthermore, it was found that everolimus does not influence GLV-1 h68 replication negatively. The combinatorial treatment of the GLV-1 h68 with everolimus was slightly superior and significantly more effective than any single agent treatment to deplete tumor cells in the panel of human NET/NEC cell lines, making this combined treatment modality feasible for further investigations [113].

Another possibility for the combinatorial treatment with GLV-1 h68 could be the usage of the multi-kinase inhibitor sunitinib. The drug, which is approved for progressive pan-NET, was recently shown to augment anti-tumor properties of an oncolytic VACV mpJX-594 (mouse-prototype JX-594) in a mouse model of progressive pan-NETs [121]. This is explained by multiple mechanisms such as the suppression of viral resistance, the increased leakiness of tumor vasculature, and, therefore, more effective viral infection and increased CD8⁺ T-cell recruitment [121]. Moreover, sunitinib was shown to be a favorable combinatorial partner for virotherapeutics-oncolytic reovirus in a murine renal cell carcinoma model [122].

Overall, the *in vitro* and *in vivo* results for all the above-mentioned OV are reasonably encouraging but require further evaluation in animal models and more realistic human tumor models, such as human NEN-derived organoids and then in clinical trials on GEP-NENs. Also, because the efficacy OV as single agents was reported as limited in cancer clinical trials [79, 123] and not enough to effectively eliminate tumors, a combination of OV with other anticancer agents should be tested to overcome this limitation.

Making Cold Tumors Hot: Immune Checkpoint Inhibitor–OV Combination Therapy: Future Perspectives for GEP-NENs

Response to PD-1 inhibition is highly correlated with the presence of CD8⁺ T cells at the invasive margin and within the tumor lesions, which define the so-called in-

flamed “hot” tumors [124]. However, most GEP-NENs patients, like many other cancer patients, are resistant to PD-1/PD-L1 blockade monotherapy or show only marginal benefit [79, 125].

Over the past 2 decades, OV have emerged as promising immunotherapeutic agents against advanced cancers. Many OV have demonstrated modest antitumor efficacies with tolerable toxicity profiles as monotherapy when administered through either intratumoral or systemic routes in clinical trials. The primary problem in oncolytic virotherapy is the risk of uncontrolled replication *in vivo* and possible transmission to patients’ contacts, such as other patients and health-care workers [126]. Moreover, there are still many issues to solve to optimize OV-based immunotherapy. These include viral species, delivery platforms, intratumoral viral spread, neutralizing antibodies, and dosing strategies [77, 127, 128]. Antiviral immunity in the host immune system is also continuously trying to clear OV within the TME [129]. Future studies should increasingly focus on the specific mechanism of the interaction between OV and the human immune system and the immune TME. This would prevent adverse events from the source of these viruses and latent virus infection and virus shedding and transmission, which would result in overall improved patient safety [81]. With this purpose, researchers seek to generate genetically modified OV, produce new delivery systems, combine different routes of delivery to enhance oncolytic virotherapy efficacy, and develop combination therapies with other therapeutic modalities, such as with ICIs [64, 79].

There is a strong mechanistic rationale for using a combination of OV and ICIs (Fig. 1). OV possess the potential to offer a simpler *in situ* vaccination approach to activate T-cell responses by locoregional immune activation, immunogenic oncolytic tumor cell death, mutant neoantigen release, and presentation and alteration of the immunosuppressive TME [83, 130, 131]. Thus, it serves as an ideal immunologic platform to potentiate and expand the antitumor efficacy of ICIs [127, 132]. Viral infection also increases the expression of CTLA-4, PD-1, and induces upregulation of PD-L1 in malignant cells [83] and on immune cells [133]. Therefore, it can prime cancer cells with low basal PD-L1 expression and render them sensitive to anti-PD-1/PD-L1 antibody therapy [134]. On the other hand, the same combination strategy may also overcome tumor resistance to oncolytic immunotherapy resulting from the reactive upregulation of PD-L1 expression in the TME, which can block T-cell activation against tumor [133, 135]. Therefore, the tandem

use of oncolytic virotherapy and PD-1/PD-L1 signaling axis inhibition can potentially alleviate what would otherwise be a dampened antitumor immune response to each of the mentioned agents [125, 136, 137] (shown in Fig. 1).

Recently, the combination of OV's with ICIs has been intensively investigated in many clinical trials. OV's encompass a diverse array of viruses and use either unmodified or modified OV's armed with cytokines and chemokines. The combination has demonstrated promising therapeutic efficacies for metastatic or unresectable tumors, in particular melanomas [17, 18, 138]. T-VEC is leading this promising combination immunotherapy [79]. The promising results from both preclinical and clinical studies [136] might represent the rationale base also to test this combination in patients with nonresponding, metastasized, advanced GEP-NENs. However, the clinical guidelines and the investigational context for administering oncolytic virotherapy, in general, remain unclear. In addition, the specific choice of which route of delivery is made without clear standards or criteria; it is mainly selected to reduce adverse events and enhance efficacy depending on the tumor sites and the objective of studies [81]. Things are even more unclear in the case of patients with GEP-NENs due to the lack of clinical studies. However, the best application of oncolytic virotherapy (combined or not with ICIs) is one related to personalized medicine; for example, in the setting of NENs of GEP origin, specific viral delivery via the hepatic artery to liver metastasis forms a one-to-one precision therapeutic mode. This may indicate the direction of oncolytic virotherapy development. The application of OV's as carriers for both tumor precision imaging and radiotherapy may also be an interesting subject of future investigation in NENs and GEP-NENs [82].

Conclusion

The success of ICIs has propelled oncology to explore other immunotherapeutic mechanisms to abrogate cancer growth. However, the accumulating data indicate that, with rare exceptions, PD-1 inhibitor monotherapy is minimally active in NENs originating outside of the lung or skin, regardless of tumor grade or differentiation. Still, ICIs might be a valid treatment option for high-grade, POD GEP NENs. In contrast, for low and intermediate graded, well-differentiated GEP-NENs, immunotherapy is still far from routine clinical application.

Even though the clinical data are lacking, the preclinical studies indicate that OV's might improve the therapeutic outcome in metastatic GEP-NENs. Oncolytic virotherapy is a safe form of immunotherapy that the anti-PD-1/PD-L1 therapy can enhance. Thus, it can become the most potent therapeutic strategy in cancer treatment if the current hurdles are to be overcome. The most significant advantage we have in harnessing oncolytic viral therapy lies in our capability to bioengineer these nanomicrobes. With an improved understanding of molecular pathophysiology, we have the potential to manipulate OV's to match evolving cancer challenges [83]. And finally, future research will, therefore, have to focus on the majority of immunologically silent or tolerant GEP-NENs with a limited expression of PD-L1 and T cell infiltration and on remodeling the host immune system and the TME – to develop a more effective treatment strategy. This probably can best be achieved by novel strategies exploring synergistic combinations and multiple combinations of ICIs like dual CTLA-4 and PD-1/PD-L1 blockade, combination with OV's or antiangiogenic agents, or combination with conventional anticancer treatments including radiotherapy, chemotherapy, and PRRT [139]. Furthermore, given that immunosuppressive TMEs can vary significantly, future efforts should also focus on finding predictive biomarkers that can help identify GEP-NEN patients who will respond best to immunotherapy and which combinations will be most effective.

Statement of Ethics

The authors have no ethical conflicts to disclose.

Conflict of Interest Statement

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