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Immunotherapy: Pancreatic Cancer and Extrahepatic Biliary Tract Cancer

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) and extrahepatic biliary tract cancer (BTC) are among the malignancies with the highest morbidity and mortality. Despite increasing knowledge on biology and novel therapies, outcome remains poor in these patients. Recent progress in immunotherapies created new hopes in the treatment of PDAC and extrahepatic BTC. Several trials tested immunotherapies in various therapeutic situations as monotherapies or in combinations. Although responses were seen in some of the trials, the value of immunotherapy in PDAC and extrahepatic BTC remains unclear in the current situation, especially regarding the complex biological characteristics with a high stroma component, intrinsic resistance mechanisms and an immunosuppressive, hypoxic microenvironment. These major hurdles have to be taken into account and overcome if immunotherapies should be successful in these tumor entities. Thereby, combinational approaches that allow on the one hand targeted therapy and on the other restore or boost the function of immune cells are promising.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal malignancies with a devastating 5-year survival rate of approximately 8% for all stages combined [1]. At early stages, pancreatic cancer is usually asymptomatic whereas at advanced stages presenting symptoms may include jaundice, abdominal pain, weight loss, steatorrhea and new-onset diabetes. Due to its silent nature in early stages, PDAC is often diagnosed at an advanced or metastatic stage.

Surgical resection at best in combination with adjuvant systemic chemotherapy is the only potentially curative treatment, but less than 20% of patients are eligible for surgery with curative intent at the time of primary diagnosis. A recently published randomized phase III trial (PRODIGE 24) showed a significant survival benefit from adjuvant modified FOLFIRINOX (mFOLFIRINOX: 5-fluorouracil [FU]/leucovorin/irinotecan/oxaliplatin) compared to standard of care therapy with gemcitabine. The overall survival rate at 3 years was 63.4% in the mFOLFIRINOX group compared to 48.6% in the gemcitabine group, however at the expense of a higher toxicity [2]. However, not all patients can receive adjuvant treatment due to postoperative morbidity or prolonged convalescence. The strategy of adjuvant chemotherapy following surgery is currently challenged by several ongoing clinical trials of neoadjuvant or perioperative chemotherapy concepts in patients with borderline resectable tumors in order to increase the R0 resection rate and consecutively the survival rate. Gemcitabine has been the standard of care for locally advanced or metastatic PDAC patients since 1997. However, over the last few years the therapeutic landscape developed with implementation of two efficacious regimens in the management of advanced PDAC. The results from the MPACT trial demonstrated an improvement in median overall survival from 7 to 8.5 months for the combination of gemcitabine and nab-paclitaxel when compared to gemcitabine alone [3]. The results of the PRODIGE 4/

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Table 1. Results from clinical trials with checkpoint inhibitors in pancreatic cancer and extrahepatic biliary tract cancer

Molecule	Regimen	Phase	Patient population	Results	Reference
Ipilimumab (anti-CTLA-4)	Monotherapy	II	Locally advanced or metastatic PDAC, *n* = 27	No objective response (1 delayed response after initial progression)	Royal et al. [18]
	Combination with gemcitabine	Ib	Advanced PDAC, *n* = 16	2 PR, 5 SD	Kalyan et al. [20]
Tremelimumab (anti-CTLA-4)	Combination with gemcitabine	I	Metastatic PDAC, *n* = 34	2 PR	Aglietta et al. [19]
BMS-936559 (anti-PD-L1)	Monotherapy	I	Multiple entities, advanced or metastatic PDAC, *n* = 14	No objective response	Brahmer et al. [23]
	Combination with chemotherapy	Ib/II	Multiple entities, metastatic PDAC, *n* = 11	3 PR, 8 SD	Wiess et al. [25]
Nivolumab (anti-PD-1)	Combination with nab-paclitaxel ± gemcitabine	I	Locally advanced or metastatic PDAC, *n* = 17	5 PR, 7 SD	Wainberg et al. [26]

CTLA-4, cytotoxic T lymphocyte-associated protein 4; PDAC, pancreatic ductal adenocarcinoma; PR, partial remission; SD, stable disease; PD-L1, programmed cell death ligand-1; PD-1, programmed cell death-1.

ACCORD 11 have proven the FOLFIRINOX regimen to be superior to gemcitabine alone with an extended median overall survival of 11.1 months, however with an increased toxicity [4]. Results from the NAPOLI-1 trial have recently shown that nanoliposomal irinotecan in combination with 5-FU and leucovorin extends survival in patients with metastatic PDAC (mPDAC) who had previously received gemcitabine-based therapy. Another option for second-line therapy is a combination of oxaliplatin with 5-FU/folinic acid (OFF) as the phase III trial CONKO-003 has demonstrated [5]. For the first time, sequential treatment algorithms are proposed based on available data for patients with mPDAC. However, despite the advances in systemic cytotoxic chemotherapy strategies, the overall survival benefit is modest, and the prognosis of mPDAC still remains dismal. An even worse situation regarding limited evidence-based systemic treatment options is found in advanced extrahepatic biliary tract cancer (BTC) with platin (cisplatin, oxaliplatin)/gemcitabine as being the only standard of care therapy [6, 7].

PDAC and extrahepatic BTC are associated with high morbidity and mortality, and there is an urgent need for novel treatment options besides chemotherapy to improve survival outcomes and quality of life. An innovative treatment modality is immunotherapy which aims at augmenting the body's own immune system to fight cancer cells. Cancer immunotherapy has experienced a breakthrough in various cancer types and revolutionized traditional cancer treatment in various entities. The rapidly growing field of immunotherapies includes immune checkpoint blockade therapies, cancer vaccinations and chimeric antigen receptor (CAR) T-cell therapies. This review aims to provide an overview on the current state of immunotherapies in PDAC and extrahepatic BTC and to give an outlook on future directions.

Checkpoint Inhibition as a New Therapeutic Strategy

The immune system has the ability to recognize and eliminate cancer cells [8]. CD8+ cytotoxic T cells identify tumor-specific antigens that are presented by the major histocompatibility complex (MHC) class I molecule expressed on antigen-presenting cells through their T-cell receptor.

Immune checkpoints are pathways that regulate the duration and amplitude of immune responses in physiological conditions in order to maintain self-tolerance and prevent autoimmunity [9]. Utilization of these pathways is an important mechanism of immune evasion of cancer cells. Tumor cells exploit immune checkpoints to avoid recognition and elimination by the immune system. The immune checkpoints that have been intensively studied are mainly cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and programmed cell death-1 (PD-1) (Table 1). CTLA-4 and PD-1 are co-inhibitory receptors expressed on the surface of T cells that function as negative regulators of T-cell activation. Monoclonal antibodies targeting these immune checkpoint regulators can enhance endogenous antitumoral activity. So far, inhibition of immune checkpoints has been shown to be particularly effective in several malignancies such as melanoma [10], non-small cell lung cancer [11], urothelial carcinoma [12], renal-cell carcinoma [13], head and neck cancer [14] and hepatic cancer [15].

Inhibition of the CTLA-4 Pathway

CTLA-4 is a co-inhibitory receptor, whereas CD28 is a co-stimulatory receptor expressed on activated CD4+ and CD8+ T cells. CTLA-4 and CD28 compete in binding the

ligands B7-1 (also known as CD80) or B7-2 (also known as CD86) on antigen-presenting cells. CTLA-4 attenuates the activity of T cells by outcompeting CD28 in binding CD80 and CD86 and delivering inhibitory signals to the T cell [9, 16]. Blockade of CTLA-4 has been shown to induce antitumoral activity [17]. Ipilimumab, a fully humanized IgG1 monoclonal antibody, blocks the ligand-receptor interaction of B7-1/B7-2 and CTLA-4. In 2010 ipilimumab was tested in a phase II trial in patients with advanced PDAC suggesting that single-agent ipilimumab does not demonstrate significant activity in the treatment of advanced PDAC [18]. A phase I dose escalation trial of tremelimumab, a fully humanized IgG2 monoclonal antibody antagonizing CTLA-4, demonstrated a safe profile when combined with gemcitabine in chemotherapy-naïve patients with metastatic PDAC [19]. A phase Ib trial of ipilimumab in combination with gemcitabine in advanced pancreatic cancer confirmed tolerability. However, the objective response rate did not seem to be significantly improved over gemcitabine alone in both trials [20].

The PD-1/PD-L1 Pathway

PD-1 is a co-inhibitory receptor expressed on T cells, B cells, monocytes and natural killer T cells [21]. PD-1 has two ligands (PD-L1 and PD-L2) that are expressed on antigen-presenting cells. Binding of PD-L1 or PD-L2 to PD-1 downregulates the expression of anti-apoptotic molecules and attenuates T cell activation [22]. Anti-PD-1 inhibitors block the interaction with PD-L1 and PD-L2 resulting in decreased tumor growth.

In a phase I clinical trial of anti-PD-L1 (BMS-936559) therapy in advanced pretreated solid tumors, no antitumor activity was seen in the 14 PDAC patients included. Other solid tumors like melanoma, lung cancer and renal-cell cancer did however show significant tumor regression [23]. Preclinical data from murine transplant models showed an antitumoral effect for PD-1 or PD-L1 blockade combined with chemotherapy [24]. A phase Ib trial evaluated pembrolizumab, a humanized IgG4 monoclonal antibody against PD-1, combined with various chemotherapies across multiple advanced solid tumors. In total 11 patients with metastatic PDAC (after first-line chemotherapy or treatment naïve) received chemotherapy in combination with pembrolizumab. Two patients showed a partial remission, 6 patients had a stable disease [25]. Interim results from a phase I trial combining nivolumab plus nab-paclitaxel with or without gemcitabine showed a response in 12 out of 17 patients (5 patients partial remission, 7 patients stable disease) with locally advanced or metastatic PDAC [26]. These results appear promising but larger clinical trials are needed to evaluate any statistically significant clinical benefit.

Regarding biliary tract cancer, encouraging results have recently been published. Thirty-four patients who had progressed on at least one line of systemic therapy received nivolumab. Out of 29 evaluable patients, 5 patients achieved partial remission and 11 patients achieved stable disease [27]. Phase II trials with mono or dual checkpoint inhibition or combined with gemcitabine/cisplatin are currently recruiting (e.g. NCT03101566, NCT02829918).

Immunogenic Subtypes of PDAC and Extrahepatic BTC

A small subset of PDAC patients with mismatch repair deficiency (MMR) showed significant clinical benefit with immune checkpoint inhibitors. In a phase II study conducted by Le and Durham [28], 86 patients with MMR-deficient advanced tumors of 12 different tumor types demonstrated efficiency with anti-PD-1 therapy. Eight patients with PDAC were included in the study and achieved an objective response rate of 62%. The mismatch repair system corrects DNA damage introduced into microsatellites (short tandemly repeated sequences) during replication to maintain genomic stability [29]. Defects in the MMR system or loss of function of MMR proteins (MLH1, MSH2, MSH6, PMS2) lead to an accumulation of mutations in microsatellites, resulting in a microsatellite instable (MSI) phenotype [30]. Unfortunately, MSI is a rare event that only occurs in about 1% of all PDAC tumors and in about 5–13% of extrahepatic BTC [31–33]. However, National Comprehensive Cancer Network guidelines encourage to consider MSI and/or MMR testing on tumor tissue for patients with locally advanced or metastatic PDAC [34].

Generally tumor immunogenicity is caused by the degree of structural epitope differences compared to normal cells that allows T cell recognition and interference [35]. A defective DNA repair system increases the tumor mutational burden (TMB) resulting in a higher neoantigen load. Thereby the increased neoantigen expression further sensitizes to immune checkpoint blockade irrespective of the tumor type. High TMB is positively correlated with response to checkpoint inhibition across diverse tumor entities [36, 37]. But compared to other solid tumors PDAC has a low TMB with a median number of about 1 somatic mutation per megabase as opposed to solid tumors with a high mutational load of about 10 somatic mutations per megabase (melanoma, lung cancer, bladder cancer) [38]. Tumor-infiltrating lymphocytes (TILs) are crucial in the complex immunogenic response and increased in high TMB [39]. But within the pronounced desmoplastic stromal compartment TILs are rare and classify PDAC more or less as noninflamed [40, 41]. Im-

munosuppressive cells like T regulatory cells or myeloid cells are frequently found in the stroma and are contradictory to TILs [42]. Additionally, the stroma-related hypoxic tumor microenvironment favors these non-TIL cells in response to activated cancer-associated fibroblasts [43–45]. In sum, the previously mentioned factors contribute to the immunosuppressive tumor microenvironement that enables a therapy resistance mechanism as a possible explanation for the unresponsiveness to checkpoint inhibition. As a consequence, pro-immunogenic strategies are needed in PDAC, as far as various tumor-associated antigens (TAAs) are known. Similar conditions are found in extrahepatic biliary tract cancer. Single agent immune checkpoint inhibitors have only very limited efficiency in both cancer entities unless the tumor is microsatellite instable and so there is a need for combination strategies.

Adoptive T-Cell Therapy

CAR T-cell therapy is a form of immunotherapy that redirects patients' T cells to specifically target and destroy tumor cells. Adoptive T-cell therapy has demonstrated impressive results in hematological B cell malignancies like B cell lymphoma [46–49] and acute lymphoblastic leukemia [46, 50].

As a first step in the manufacturing process, T cells are isolated from peripheral blood of the patient by means of leukapheresis. Ex vivo T cells are activated and virally transducted with the vector encoding the CAR, an artificial T cell receptor. CAR T cells are further extended and reinfused into the patient. CAR T cells recognize tumor surface antigens independently from MHC restriction and kill tumor cells upon antigen contact.

Multiple early phase I studies demonstrate efficacy in CAR T-cell therapy in preclinical models of pancreatic cancer. Targeting against the tumor antigen mesothelin [51, 52], carcinoembryonic antigen [53], prostate stem cell antigen [54, 55], HER2 neu, CD24 [56], CD133 and mucin-1 (MUC-1) [57] has shown activity in preclinical tumor models. Currently, multiple clinical phase I and II trials using CAR T-cell therapy targeting prostate stem cell antigen (e.g. NCT02744287) are ongoing.

CAR T-cell therapy has made tremendous successes in hematological malignancies, its application in PDAC and extrahepatic BTC is still at the beginning.

Vaccination Strategies in PDAC and Extrahepatic BTC

A key characteristic of PDAC is its low immunogenicity that is driven by various means. As mentioned before, TMB is low in PDAC and extrahepatic BTC with only rarely expressed neoantigens, limiting the response to immunotherapies [37, 38]. Pro-immunogenic strategies are warranted to overcome this hurdle as far as various TAAs are known for both cancers, especially the highly immunogenic neoantigens driven by KRAS mutations in PDAC [58]. Particularly immune modulation via vaccination strategies seems to be promising in both cancer types. Thereby, vaccine immunotherapy in cancer treatment aims to activate an immune response by achieving TAA presentation. Therefore, different concepts exist including whole-cell, dendritic-cell (DC), DNA and peptide vaccines.

Peptide and Protein-Based Vaccines

Various TAAs are described in PDAC and BTC that can be targeted, like mucin 5AC [59], C-ERC/mesothelin [60, 61], mutS homolog 2 (MSH2), postmeiotic segregation increased 1 (PMS1) [62], cancer-testis antigens [63] and Forkhead box M1 [64]. In line a phase I study obtained first interventional steps with an autologous vaccine HSPPC-96 (heat shock protein gp96) purified from completely resected PDACs. In total 10 patients received each 4 doses of autologous HSPPC-96. Safety was proved, and no correlation between immune response and prognosis could be seen [65].

KRAS has been in the focus as the key TAA knowing that in up to 90% of PDAC KRAS point mutations are found (most frequent $KRAS^{G12D}$) that can allow cytotoxic T cell recognition [66–69]. Initial trials with RAS vaccines could verify safety and showed a transient T cell PDAC response [70]. Following that, two phase II clinical trials used subcutaneous KRAS vaccination combined with GM-CSF in the adjuvant setting [71, 72]. In total 23 patients were treated, 10 patients with a single peptide vaccine that corresponded to the KRAS mutation of the cancer and 13 patients with a mixture of the 7 most common mutated RAS peptides in PDAC. Vaccination was well tolerated, and survival times after 10 years were 20% (4/20 evaluable patients) compared to 0% (0/87) in a cohort of nonvaccinated patients treated in the same period [71, 72]. Similar results were achieved in another adjuvant use of a RAS peptide vaccine in 5 PDAC patients with a mean overall survival (OS) time of 44.4 months [23]. Finally Abou-Alfa et al. [73] vaccinated 24 resected PDAC patients harboring a $KRAS^{G12D}$ mutation with a 21-mer peptide vaccine containing the KRAS mutation of the patient's tumor. The therapy was well tolerated, and 9 patients were evaluable for immune response. The median OS time in this study was 20.3 months. Currently under investigation is the combination of the RAS vaccine TG01 (a mixture of 7 RAS peptides)/GM-CSF with gemcitabine versus gemcitabine monotherapy as an adjuvant treatment in resected (R0 or R1) RAS-mutant PDAC patients (NCT02261714). A first interim analysis

of 19 patients showed promising results with a 2-year OS rate of 68.4% and a median OS of 33.1 months [74]. However, these promising results in the adjuvant setting could not directly be transferred to the palliative setting when used as monotherapies. Gjertsen et al. [70] treated 38 patients with advanced PDAC and showed a significant survival benefit that depends on the immune response (median OS 148 days vs. 61 days in nonimmune responders; $p = 0.0002$), when using a KRAS vaccine adjusted to the patients' mutation.

Carcinoembryonic antigen is another vaccination target of interest tested in PDAC. Although showing promising results in early phase clinical trials [75, 76], a randomized phase III trial with a carcinoembryonic antigen-targeted vaccine in PDAC second-line therapy failed to show a survival benefit compared to palliative chemotherapy or best supportive care [77]. The high need for a tumor response and the complex microenvironment in advanced PDAC may be more suitable for combinational approaches. Considering that, we are looking forward to the data of further upcoming randomized trials, in combination with checkpoint inhibitors (e.g., NCT02472977, NCT02350673).

Another focus is set on the antigastrin-17 diphtheria toxin-coupled vaccine G17DT that acts against the growth factor gastrin-17. A first phase II study in 30 advanced PDAC patients confirmed a dose dependent significant survival benefit for antibody responders (217 days) versus nonresponders (121 days) [78]. The treatment of 154 chemotherapy-naïve patients with advanced PDAC with G17DT led to a nearly doubled median OS time (151 days) compared to the placebo group (82 days). Again, the survival benefit was significantly dependent on the anti-G17DT response [79]. Negative results were reported by Shapiro et al. [80] in advanced PDAC treatment for the combination of gemcitabine plus G17DT (median OS 5.8 months) compared to gemcitabine plus placebo (median OS 6.6 months). The final results of a phase III trial in advanced PDAC patients treated with sequentially administered G17DT or placebo are pending (NCT02118077).

Increased telomerase expression in PDAC [81] is accompanied by the induction of human telomerase reverse transcriptase [82] and relevant for tumorigenesis. Therefore the human telomerase reverse transcriptase peptide-based vaccine GV1001 was developed. Despite promising phase I/II trial results, GV1001 failed to show a significant survival benefit for advanced PDAC patients in a phase III trial [83].

MUC-1 is a glycoprotein that has a function in cell signaling with oncogenic implications on cell polarity, motility and angiogenesis [84, 85]. Overexpression is found in several tumors like PDAC [86] and BTC [87] and is linked to a highly immunogenic target [88] with implications on drug resistance [89]. In a phase I trial 6 PDAC and 3 BTC patients in a palliative setting were treated with a 100-mer MUC-1 peptide, resulting in mainly tumor progression after 7 weeks [90]. Similar disappointing results for peptide-based MUC-1 vaccines were obtained by Ramanathan et al. [91].

Furthermore, in BTC alternative epitopes and approaches can be found. Aruga et al. treated 9 (4 intrahepatic, 3 extrahepatic BTC and 1 gallbladder cancer) advanced chemotherapy refractory BTC patients with a three-peptide vaccine (cell division cycle-associated 1, cadherin 3 and kinesin family member 20A). All patients had a peptide-specific T cell immune response, and stable disease was observed in 5 of 9 patients after 8 weeks of therapy. The median progression-free survival was 3.4 months and the median OS 9.7 months [92]. In another similar therapeutic situation 9 patients were treated with a four-peptide derived vaccine from cancer-testis antigens. Clinical responses were observed in 6 of 9 patients with a median OS of 380 days. The injection site reaction and cytotoxic T cell induction seemed to be prognostic factors for survival [93].

Whole-Cell-Based Vaccines

Whole-cell vaccines are normally derived from the primary tumor and express various epitopes of CD8+ and CD4+ T cells. This advanced approach ensures to hit all potentially relevant antigens and allows multiple TAA targeting in parallel. However, only a small number of patients undergo surgery for PDAC, and the total number of tumor cells is often low; therefore, allogeneic cell lines are alternatively used [94–97]. The use of allogeneic tumor cells can further bypass the need of individualizing each therapy. The mixture of several cell lines from various tumors can improve the overlap of the antigens expressed and the patient's tumor. Moreover, preclinical models taught us that insufficient tumor defeat is mainly caused by the inability of the immune system to appropriately respond to TAAs [98]. Here, cytokines can assist by enabling an immunological boost, and GM-CSF was found to be a highly potent inducer [99], that can also be used therapeutically [99, 100].

GVAX pancreas is a lethally irradiated allogeneic whole-cell tumor vaccine that is genetically modified to secrete GM-CSF and in parallel deliver TAAs [99]. In the ECLIPSE phase IIb trial, advanced PDAC patients who had failed previous therapy were enrolled and treated with either GVAX pancreas plus mesothelin-expressing live-attenuated *Listeria monocytogenes* (CRS-207) as an immunogenic boost, or CRS-207 alone or physician's choice of single-agent chemotherapy. Upfront the patients received low-dose cyclophosphamide for inhibition of regulatory T cells in the GVAX combination arm, an effect confirmed in BTC [101]. With a median OS of 3.8 months GVAX plus CRS-207 was neither superior to

the chemotherapy arm (4.6 months) nor the CRS-207 arm (5.4 months) [102].

Within another single-center phase II study, 60 curatively resected patients were treated with GVAX and subsequent 5-FU-based chemoradiation. The therapy was well tolerated, the median OS was 24.8 months, and the disease-free survival correlated with the induction of a mesothelin-specific T cell response in the patients [103]. The aforementioned telomerase vaccine GV1001 failed to show a survival benefit in combination with gemcitabine (median OS 5.9 months) to gemcitabine (median OS 7.3 months) only in chemotherapy-naïve, advanced PDAC patients in a phase III trial [83]. However, as far as GVAX could not fulfill the expectation of a one-fits-all vaccine, alternative strategies are needed to boost the efficacy. Therefore combinations of GVAX with checkpoint inhibitors like nivolumab (NCT02243371, NCT03190265), pembrolizumab (NCT02648282) or ipilimumab (NCT-03190265) are currently under investigation.

Another tested vaccine is algenpantucel-L consisting of allogeneic irradiated pancreatic cancer cells with expression of α-1,3-galactosyl transferase, an enzyme catalyzing the synthesis of α-galactosyl epitopes. The vaccine thereby aims to boost the activity against α-galactosyl-labeled tumor cells. Promising preclinical results [104, 105] paved the way for clinical trials. The IMPRESS phase III study included 722 resected PDAC patients who received adjuvant standard of care chemotherapy with or without radiation with algenpantucel-L. The study failed by showing a median OS of 27.3 months for the addition of algenpantucel-L compared to 30.4 months with standard of care alone [106].

Dendritic Cell Vaccines

DCs act as highly effective antigen-presenting cells that facilitate cytolytic and regulatory T cell reaction [107]. DCs can be manipulated in several ways in order to achieve an antitumor response [108].

Within a phase I trial DCs were pulsed with a mixture of three types of Wilms tumor 1 peptides (MHC I and/or II) and combined with gemcitabine as palliative treatment in advanced PDAC patients. In this trial the combination of both MHC class I- and II-restricted epitopes was linked to a delayed-type hypersensitivity (3/10 patients) that resulted in a significant survival benefit (median OS 717 days) compared to negative control [109]. In a previous phase I study, safety for the WT1 vaccine in combination with gemcitabine could also be confirmed for intrahepatic (4 patients) and extrahepatic (4 patients) BTC, although an objective clinical efficacy was missing with a disease control rate of 50% after 2 months of therapy [110].

As previously mentioned MUC-1 is a highly immunogenic target in PDAC but also BTC. Lepisto et al. [111] conducted an adjuvant phase I/II clinical trial of a MUC-1 peptide-loaded DC vaccine in 10 resected PDAC and 2 BTC patients. After 4 years, 33% (4/12) of the patients were still alive without evidence of a relapse [111]. Feasibility of the approach is confirmed in another 10 cancer patients treated with autologous DCs transfected with cDNA of MUC-1 by Pecher et al. [112].

A retrospective analysis of 65 advanced BTC patients that were treated with WT1 and/or MUC1 pulsed DCs verified the safety of the vaccination and showed a clinical response in patients who underwent additional chemotherapy (median OS with chemotherapy 8.2 months, without chemotherapy 5.3 months) [113].

Discussion

The recent immunotherapy progress in solid oncology created a hype and new hopes in various cancer entities. However, we had to learn that this could not be generalized, and tumor response is entity specific. Definitive positive results are published for MSI-high tumors and for tumors with a high mutational burden. Regarding this in MSI-high tumors checkpoint inhibitor therapy is FDA approved irrespective of the entity. The evolving field of checkpoint inhibition shows no clear benefit at the moment in phase I–II trials; anyhow, there are some responders. For now, the interpretation of the published data is hampered by low patient numbers, partially missing controls, varying therapy lines/previous therapies and inconsistent therapy regimen. Only few studies have used the state-of-the-art first-line therapies for advanced PDAC, e.g. with gemcitabine/nab-paclitaxel combined with nivolumab. The optimal time point for the use of a checkpoint inhibitor remains elusive. Anyhow combinational strategies of a checkpoint inhibitor with a chemotherapy backbone seem to be more promising in PDAC and extrahepatic BTC. Probably this is caused by the special biology with a high stroma component, intrinsic resistance mechanisms and an immunosuppressive and hypoxic microenvironment. PDACs are among the most immune cold cancers with a low mutational burden. Both are contradictory to the aforementioned criteria for response to checkpoint inhibitors. Moreover, there are no established biomarkers that predict therapy response in case of checkpoint inhibition in PDAC and extrahepatic BTC, but in some studies a high PD-L1 expression was associated with poor outcome [114].

Regarding all those tumor microenvironment-driven factors it is difficult to believe that immune checkpoint inhibitor monotherapy will be successful at least in PDAC. To overcome these hurdles alternative strategies are warranted that activate the immune response. A possible approach could be vaccination strategies. However, after first promising results GVAX as the most intensively studied

vaccine failed in a phase III trial. This shows the need for a better stratification of the patients and the use of alternative combinational strategies as an example with immune checkpoint inhibitors or other immune modulators.

Statement of Ethics

The authors have no ethical conflicts to disclose.

Disclosure Statement

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Author Contributions

A.K.B., T.J.E. and L.P. were responsible for conception, literature research, writing and drafting of the manuscript.

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