Challenges and perspectives for immunotherapy in oesophageal cancer: A look to the future (Review)

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Abstract. Oesophageal cancer is one of the most aggressive malignancies with limited treatment options, thus resulting in a high morbidity and mortality. With 5-year survival rates of only 5-10%, oesophageal cancer holds a dismal prognosis for patients. In order to improve overall survival, the early diagnosis and tools for patient stratification for personalized treatment are urgent needs. A minority of oesophageal cancers belong to the spectrum of Lynch syndrome-associated cancers and are characterized by microsatellite instability (MSI). Microsatellite instability is a consequence of defective mismatch repair protein functions and it has been well characterized in other gastrointestinal tumours, such as colorectal and gastric cancer. In the latter, high levels of MSI are associated with a better prognosis and with an increased benefit to immune-based therapies. Therefore, similar therapeutic approaches could offer an opportunity of treatment for oesophageal cancer patients with MSI. Apart from immune checkpoint inhibitors, other immunotherapies such as adoptive T-cell transfer, peptide vaccine and oncolytic viruses are under investigation in oesophageal cancer patients. In the present review, the rationale and current knowledge about immunotherapies in oesophageal cancer are summarised.

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Abbreviations: B7-H1, B7-homolg1; CAR, chimeric antigen receptor; CCL, chemokine (C-C motif) ligand; CD, cluster of differentiation; CTLA-4, cytotoxic T lymphocyte-associated antigen-4 (CD152); FDA, US Food and Drug Administration; HER-2, human epidermal growth factor receptor-2; HLA, human leukocyte antigen; ICOS, inducible T-cell costimulatory molecule; IDO-1, indoleamine-2,3-dioxygenase-1; IFN, interferon; IL-18, interleukin-18; MAGE, melanoma antigen gene; MDSC, myeloid-derived suppressor cell; MHC major histocompatibility complex; MICA, MHC class I chain-related protein A; MICB, MHC class I chain-related protein B; MSI, microsatellite instability; NK, cells natural killer cells; NY-ESO-1, New York oesophageal squamous cell carcinoma-1 protein; PD-1 programmed cell death protein 1 (CD279); PD-L1, programmed cell death 1 ligand 1 (CD274); RNA, ribonucleic acid; TCR, T-cell receptor; TREGs, regulatory T-cells

Key words: immunotherapy, oesophageal cancer, immune checkpoint inhibitors, adoptive T-cell therapy, peptide vaccine, oncolytic viruses

Contents

1. Introduction

The incidence of oesophageal cancer has rapidly increased over the past years and it is currently the fifth most common type of cancer worldwide with a very high mortality rate (1,2). Oesophageal cancer is subdivided into two groups according to its histological appearance: Oesophageal squamous cell carcinoma (predominant in western countries) and oesophageal adenocarcinoma (most common form in Asia) (3,4). Thus far, no molecular markers for prognosis or treatment efficacy have been discovered for squamous cell carcinoma. For oesophageal adenocarcinoma, the human epidermal growth factor receptor-2 (HER-2) status has been proven to be an efficient biomarker. HER-2 is scored by

immunohistochemistry for protein expression or fluorescent *in situ* hybridization for HER2 gene amplification (5). If positive, a targeted therapy option with trastuzumab for HER-2 is the treatment of choice (6,7). Nevertheless, oesophageal cancer is mostly treated by radiation in combination with chemotherapy or surgery. However, the 5-year overall survival rate remains very poor and is only at 5-10% (8,9). Nonetheless, surgery is not applicable in approximately half of patients as distant metastases are already present at the time of diagnosis (10). The most commonly used chemotherapeutic agents for oesophageal cancer treatment are 5'-fluorouracil and platinum agents in combination with radiotherapy (11,12).

The major risk factors for oesophageal cancer are represented by smoking, the consumption of hot tea, red meat consumption, poor oral health, low intake of fresh fruits and vegetables, alcohol abuse, obesity, nass use (a chewing tobacco product), opium consumption and low socioeconomic status (13-24). The majority of these risk factors induce gene mutations which can be recognized by the immune system (25). In addition, a minority of oesophageal cancers belong to the spectrum of Lynch syndrome-associated cancers and are characterized by microsatellite instability (MSI) (26). Therefore, the use of immunotherapy approaches in oesophageal cancer appears to be an attractive novel therapeutic strategy.

The identified main reasons for the high mortality rate of patients with oesophageal cancer are mainly the late stage of diagnosis (13) and the key role of tumour microenvironment in this type of cancer (27), where the surrounding stromal cells seem to exert an important influence in supporting tumour cell survival (27). Apart from cancer-associated fibroblasts, that are able to support tumour growth and metastasis by altering the extracellular matrix by secreting growth factors and cytokines, several immune cells [e.g., myeloid-derived suppressor cells, tumour-associated macrophages and regulatory T-cells (TREGS)] are involved in support the development of oesophageal cancer (27). Therapies targeting the tumour microenvironment and/or the immune system may thus be able to increase the survival of patients with oesophageal cancer. Over the past years, immunotherapy in particular has revolutionized the management and outcome of several types of cancer, such as melanoma, lung, gastric and kidney cancer (28). Therefore, it may be advantageous to explore the benefits from immunotherapy for oesophageal cancer. The identification and selection of robust biomarkers predicting clinical benefit are also mandatory before commencing immunotherapy treatment, as even though generally well-tolerated compared to standard therapies, immunotherapy is associated occasionally with severe toxic side-effects, such as cutaneous, gastrointestinal, endocrine and hepatic toxicity. Thus, only patients with oesophageal cancer who have the highest likelihood of benefit from immunotherapy should be offered this therapeutic regimen. For example, it is well-established that immune checkpoint inhibitors are particularly effective against mismatch repair-deficient tumours (29). In general, tumours with MSI have a higher mutation rate, which increases the probability for the immune system to recognize tumour cells (29-31). Recently, several reviews have summarized the current knowledge on immunotherapy and cancer (32-34). The present review focuses on the current state of the use of immunotherapy in oesophageal cancer.

2. Biological background of tumour immunotherapy

The immune system is a highly complex and specialised biological network including specific cells, protein and organs and is usually composed of two types: Adaptive (specific) and innate (non-specific) (35). In recognising and preventing the spread of cancer cells, the innate immunity components, such as natural killer (NK) cells, dendritic cells and macrophages are of pivotal importance; nevertheless, T-cells from the adaptive immune are recruited in order to track and kill tumour cells (35,36). Recently, a new model that provides a mechanistic explanation of this interaction termed 'cancer-immunity cycle' has been suggested (35). According to this model, dead cancer cells release antigens that in turn are recognised by antigen-presenting cells (particularly by dendritic cells). This results in the priming and activation of dendritic cells and T-cells in lymph nodes, followed by the recruitment of helper T-cells [cluster of differentiation (CD)4^+-T-cells] and cytotoxic T-lymphocytes ($CD8^+$-T-cells) at the tumour site. Following the infiltration of the tumour microenvironment, immune cells recognize and attack tumour cells that results in the release of further tumour antigens. The whole cancer-immunity cycle is fine-tuned by different stimulating and inhibitory factors, such as chemokines, cytokines, metabolic compounds, surface proteins and immune checkpoint receptors to prevent autoimmunity (37).

Cancer cells use different strategies to escape the immune system, and to capture and reprogram immune cells, leading to immune evasion. Among these strategies is the mechanisms of shedding of MHC class I chain-related protein A and B (MICA and MICB) from tumour cells into the tumour microenvironment as protection against NK cell-mediated killing (38-40). In addition, tumour cells express immune checkpoint proteins, such as programmed cell death 1 ligand 1 (PD-L1) and receptors, such as cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) on the surface, but also secrete exosomes which contains these immune checkpoint regulators. After binding to proteins expressed on the immune cells (T-cells, B-cells and myeloid cells) the checkpoint regulators exert an inhibitory signal and lead to the suppression of the immune response (41-43).

Furthermore, cancer cells, as well as tumour-associated macrophages are able to secrete chemokines, such as chemokine (C-C motif) ligand (CCL)-17 and CCl-22 which attract a subpopulation of T-cells, the so-called TREGs. TREGs are known to regulate and suppress the activity of other immune cells and to help preventing autoimmune reactions under healthy conditions (44,45). In tumour tissue, TREGs protect cancer cells and foster tumour growth (46,47). Moreover, $CD8^+$-T-cells are inhibited directly by myeloid-derived suppressor cells (MDSCs) which are stimulated by tumour-derived growth factors (48,49). In addition, stromal cells in the tumour microenvironment inhibit the function of the immune system further supporting tumour progression and metastasis (50).

3. Immunotherapy in oesophageal cancer

The immune system is a complex network of interacting cells and biochemical signals that orchestrate the recognition and attack of external antigens, whilst preventing autoimmune reactions. Under physiological conditions, this is guaranteed by a fine-tuned interplay between immune cells and a balance between stimulatory and inhibitory signals (51,52). Cancer cells often find a way to de-regulate the balanced immune system by manipulating signalling pathways to evade from immune surveillance. To overcome the mechanisms of tumour immune evasion and use the immune system as weapon against cancer, either agonists of stimulatory receptors or antagonists of inhibitory signals can be used (41). Nevertheless, according to currently available study results, only a subset of oesophageal cancer patients may benefit from immunotherapy (53). Therefore, there is an urgent need to identify biomarkers for the prediction of the benefit from immunotherapy, so that patients can be selected for treatment and those who have no benefit from immunotherapy are spared from side-effects (e.g., cutaneous, gastrointestinal, endocrine and hepatic toxicity) and therapy failure. In light of this scenario, currently, several clinical trials are underway to evaluate the efficacy of different immunotherapies combined with other treatment options in oesophageal cancer patients (Table I) with the aim to increase the therapeutic option for oesophageal cancer patients. The majority of these studies are ongoing Phase 2 studies and the results have not been published yet.

In the following section, the main immunotherapy approaches that have been studied thus far will be discussed (Fig. 1).

4. Immune checkpoint inhibitors

Immune checkpoints are of pivotal importance to prevent autoimmunity reactions by the inhibition of antigen recognition via T-cell receptors (TCRs) (41,54,55). Cancer cells use immune checkpoint proteins to inactivate the adaptive immune system by blocking tumour specific T-cells and escape from immune surveillance. Thus far, the immune checkpoint receptors programmed cell death protein 1 (PD-1; also known as CD279) and CTLA-4 (also known as CD152) have been found to be associated with the inhibition and downregulation of T-cell activity (41,54,55).

PD-1 receptor is highly expressed on T-cells, B-cells and NK cells. The ligand for PD-1 receptor is PD-L1 often also termed B7-homolog 1 (B7-H1) or CD274. This molecule is expressed in peripheral tissues following exposure to inflammatory cytokines and limits T-cell activity (56). Furthermore, interleukin (IL)-18, an inflammatory cytokine that accumulates in the tumour microenvironment, results in the upregulation of PD-L1 in activated mature NK cells and triggers immunosuppression (57). In melanoma, lung, breast, pancreatic, gastric, colon, ovarian and oesophageal cancers, PD-L1 is often found overexpressed on cancer cells (58). This enables tumour cells to interact with PD-1 receptors on T-cells and this interaction prevents T-cell activation, proliferation and ultimately leading to T-cell apoptosis (41).

The expression of CTLA-4 receptor is restricted to activated T-cells (e.g., TREGs), whereas the homolog CD28 is also expressed on non-activated T-cells. Ligands for both receptors are the immunoglobulin proteins B7-1 (CD80) and B7-2 (CD86), which are expressed early during the immune response on antigen-presenting cells, such as macrophages and dendritic cells or on B-cells and monocytes, respectively. CTLA-4 receptor has a higher affinity for ligands and competing with CD28 on ligand binding; the interaction between B7-1 or B7-2 with CD28 results in T-cell activation, whereas the interaction with CTLA-4 inhibits T-cell activation at an early stage (59,60).

It has been widely proven that PD-L1 expression is one of the key mechanisms through which several cancers evade the immune response; thus, it is not surprising that inhibitors of PD-L1 and PD-1 have been identified thus far as one of most efficient and broadly used immunotherapies for cancer (61-71). Recently, a monoclonal antibody targeting PD-1, pembrolizumab, has been approved for the treatment of oesophageal and oesophago-gastric junction adenocarcinoma by the US Food and Drug Administration (FDA) (8). The prerequisite for the treatment of oesophageal cancer with pembrolizumab is either a proven PD-L1 expression on the cancer cells and a high MSI, or a proven defective mismatch repair system. Therefore, most probably, the subgroup of Lynch syndrome-associated oesophageal cancers patients may benefit from this new treatment option. According to a previous study, it is possible to predict the efficacy of pembrolizumab in patients with oesophageal cancer by using a six-gene interferon-γ gene expression signature (72). This offers the possibility to stratify oesophageal cancer patients and limit the targeted treatment to the group that will most probably benefit from the anti-PD-L1 therapy.

Earlier in 2020, the FDA approved nivolumab, a fully human monoclonal antibody against PD-1 (73) for patients with unresectable advanced, recurrent or metastatic oesophageal squamous cell carcinoma as a second line following 5'-fluorouracil- and platinum-based chemotherapy. The overall survival benefit is 2.5 months according to a phase 3 clinical study (74).

Currently, combination therapies with anti-PD1 and anti-CTLA-4 antibodies are forthcoming (75). According to the first preliminary results from clinical studies (NCT02743494, CheckMate 648 and CheckMate 649) the combination of nivolumab with the anti CDLA-4 antibody, ipilimumab, led to an improved clinical response in oesophageal cancer compared to treatment with nivolumab alone (76,77). The combination of nivolumab and ipilimumab appears to be safe; nevertheless, it must be considered that CTLA-4 blockade results in more severe and more common side-effects than it is the case for targeting PD-1/PD-L1 alone. Therefore, the development of novel strategies for reducing serious adverse side-effects is an urgent need and the first steps need to be carefully controlled (78).

As a potential biomarker for prediction of the response to immune checkpoint inhibitor therapy, the total amount of $PD\text{-}1^+$ $CD4^+$ T-cells in the tumour microenvironment is discussed. According to the presence or absence of $CD4^+$ T-cells and PD-1 expression in the tumour microenvironment, a stratification of patients is possible. The absence of $CD4^+$ T-cells and PD-1 expression results in immunological ignorance; in a situation where only one component (either $CD4^+$ T-cells or PD-1)

Table I. Clinical trials investigating immunotherapeutic options in combination with other therapeutic options in oesophageal cancer patients.

Immuno-therapeutic option	Stage of development	Combined with	ClinicalTrials gov.identifier	Start time	Current status
Immune checkpoint inhibitor treatment					
Nivolumab	Phase 1/2	Chemoradiotherapy	NCT03278626	2017	Active, but not recruiting
	Phase 1/2	Chemoradiotherapy	NCT03544736	2018	Recruiting
	Phase 2	Chemoradiotherapy + Ipilimumab	NCT03604991	2018	Recruiting
	Phase 2	Chemoradiotherapy + Ipilimumab	NCT03437200	2018	Recruiting
	Phase 3	Ipilimumab + Chemotherapy	NCT3143153	2017	Recruiting
	Phase 2	Ipilimumab	NCT03416244	2018	Recruiting
	Phase 1/2	Ipilimumab + INCAGN01949	NCT03241173	2017	Completed in 2020, but no results published
	Phase 1	Chemoradiotherapy + Relatlimab	NCT03044613	2017	Recruiting
	Phase 2	Chemoradiotherapy + Cetuximab	NCT04229459	2020	Recruiting
	Phase 1	Chemotherapy	NCT03914443	2019	Recruiting
	Phase 3	After chemotherapy	NCT02569242	2015	Active, but not recruiting
	Phase 3	After chemoradiotherapy and surgery	NCT02743494	2016	Active but not recruiting
	Phase 1	+ Rucaparib	NCT03995017	2019	Recruiting
	Phase 1	+ Mogamulizumab	NCT02946671	2016	Completed in 2020, but no results published
	Phase 1	+ Mogamulizumab	NCT02476123	2015	Completed in 2020, but no results published
	Phase 1/2	Radiotherapy	NCT03544736	2018	Recruiting
Pembrolizumab	Phase 2	Chemoradiotherapy	NCT02844075	2016	Active, but not recruiting
	Phase 2	Chemoradiotherapy	NCT03064490	2017	Recruiting
	Phase 2	Chemoradiotherapy	NCT03322267	2017	Recruiting
	Phase 1	Chemoradiotherapy	NCT03792347	2019	Active, but not recruiting
	Phase 2	Chemoradiotherapy + chemotherapy	NCT02998268	2016	Active, but not recruiting
	Phase 2	After chemoradiotherapy and surgery	NCT02844075	2016	Active but not recruiting
	Phase 2	After chemoradiotherapy and surgery	NCT03322267	2017	Recruiting
	Phase 2	Chemotherapy + Trastuzumab	NCT02954536	2016	Recruiting
	Phase 2	+ Epacadostat	NCT03592407	2018	Withdrawn due to safety concerns
	Phase 3	Chemotherapy	NCT03189719	2017	Active, but not recruiting
	Phase 3	Chemotherapy	NCT03881111	2019	Withdrawn due to protocol amendment
	Phase 2	Chemotherapy	NCT04437212	2020	Recruiting
	Phase 2	Radiotherapy	NCT02830594	2016	Active, but not recruiting
	Phase 1	Brachytherapy: 16Gy/2F	NCT02642809	2015	Active, but not recruiting
	Phase 1	DKN-01	NCT02013154	2013	Active, but not recruiting
	Phase 1/2	INCAGN01876 + Epacadostat	NCT03277352	2017	Completed in 2020, but no results published

Table I. Continued.

Immuno-therapeutic option	Stage of development	Combined with	ClinicalTrials gov.identifier	Start time	Current status
Immune checkpoint inhibitor treatment					
	Phase 2	Tadalafil	NCT03993353	2019	Recruiting
	Phase 2	+ CRS-207	NCT03122548	2019	Terminated because of low enrolment and lack of clinical activity in other CRS-207 studies
Camrelizumab	Phase 2	Radiotherapy	NCT03200691	2017	Recruiting
	Phase 2	Radiotherapy	NCT03187314	2017	Recruiting
	Phase 2	Chemotherapy	NCT03917966	2019	Not yet recruiting
	Phase 3	Chemotherapy	NCT03691090	2018	Recruiting
	Phase 2	Chemoradiotherapy	NCT04390945	2020	Recruiting
	Phase 3	Chemoradiotherapy	NCT04426955	2020	Recruiting
	Phase 3	Chemoradiotherapy	NCT04404491	2020	Recruiting
	Phase 2	After chemoradiotherapy	NCT03817658	2019	Not yet recruiting
	Phase 1	After chemoradiotherapy	NCT03985046	2019	Recruiting
	Phase 2	After chemoradiotherapy	NCT04286958	2020	Recruiting
	Phase 2	Apatinib	NCT03736863	2019	Not yet recruiting
	Phase 2	Apatinib + chemotherapy	NCT03603756	2018	Recruiting
	Phase 2	Nimotuzumab	NCT03766178	2018	Recruiting
Sintilimab	Phase 1/2	Chemotherapy	NCT03946969	2019	Recruiting
	Phase 3	Chemotherapy	NCT03748134	2018	Recruiting
	Phase 1	Chemoradiotherapy	NCT03940001	2019	Recruiting
Spartalizumab	Phase 1	LGK974	NCT01351103	2011	Recruiting
	Phase 1/2	LAG525	NCT02460224	2015	Active, but not recruiting
	Phase 2	LAG525	NCT03365791	2017	Active, but not recruiting
	Phase 2	MCS110	NCT03785496	2018	Active, but not recruiting
	Phase 1	TNO155	NCT04000529	2019	Recruiting
Tislelizumab	Phase 2	Chemotherapy	NCT03469557	2018	Active, but not recruiting
	Phase 3	Chemotherapy	NCT03783442	2018	Recruiting
	Phase 3	Chemoradiotherapy	NCT03957590	2019	Recruiting
Toripalimab	Phase 2	Chemotherapy	NCT03985670	2019	Recruiting
	Phase 3	Chemotherapy	NCT03829969	2019	Recruiting
	Phase 2	Chemoradiotherapy	NCT04006041	2019	Recruiting
	Phase 2	Chemoradiotherapy	NCT04005170	2019	Recruiting
	Phase 2	Chemoradiotherapy	NCT04084158	2019	Recruiting
	Phase 2	Chemoradiotherapy	NCT04177875	2019	Recruiting
	Phase 2	After Chemoradiotherapy + surgery	NCT04437212	2020	Recruiting
HLX-10	Phase 3	Chemotherapy	NCT03958890	2019	Recruiting
Avelumab	Phase 1/2	Chemoradiotherapy	NCT03490292	2018	Recruiting
	Phase 2	Chemoradiotherapy	NCT03800953	2019	Not yet recruiting
	Phase 2	Chemotherapy before surgery	NCT03399071	2018	Recruiting
Atezolizumab	Phase 2	Chemoradiotherapy	NCT03087864	2017	Completed in 2020, but no results published
	Phase 1	Chemoradiotherapy	NCT03784326	2018	Recruiting
	Phase 2	Chemotherapy	NCT03448835	2018	Recruiting
	Phase 1/2	Cabozantinib	NCT03170960	2017	Recruiting
	Phase 1/2	KY1044	NCT03829501	2019	Recruiting
	Phase 1/2	DKN-01	NCT04166721	2019	Recruiting

Table I. Continued.

Immuno-therapeutic option	Stage of development	Combined with	ClinicalTrials gov.identifier	Start time	Current status
Immune checkpoint inhibitor treatment					
Durvalumab	Phase 2	Chemoradiotherapy	NCT02962063	2016	Recruiting
	Phase 2	Chemoradiotherapy	NCT03777813	2018	Recruiting
	Phase 2	Chemoradiotherapy + chemotherapy	NCT02735239	2016	Active but not recruiting
	Phase 2	After chemoradiotherapy	NCT04054518	2019	Not yet recruiting
	Phase 2	After chemoradiotherapy + surgery	NCT02639065	2015	Active but not recruiting
	Phase 2	After chemoradiotherapy + surgery	NCT02520453	2015	Active but not recruiting
	Phase 2	Chemoradiotherapy + Tremelimumab	NCT03377400	2017	Active but not recruiting
	Phase 1	Chemotherapy + Tremelimumab	NCT02658214	2013	Active but not recruiting
	Phase 2	Tremelimumab	NCT03292250	2017	Recruiting
	Phase 2	Tremelimumab	NCT03982173	2019	Not yet recruiting
	Phase 2	Tremelimumab	NCT04159974	2019	Recruiting
	Phase 1/2	Tremelimumab + SBRT	NCT03212469	2017	Recruiting
SHR-1316	Phase 2	Chemotherapy	NCT03732508	2018	Recruiting
	Phase 2	Nimotuzumab	NCT03766178	2019	Not yet recruiting
Adoptive T-cell therapy					
	Phase 1	HER2Bi-armed T-cells + IL-2	NCT02662348	2016	Unknown
	Phase 1/2	CAR-T combined with PD-1 knockout T-cells	NCT03706326	2018	Recruiting
	Phase 1	CAR-T combined with CAdVEC (oncolytic adenovirus)	NCT03740256	2018	Recruiting
	Phase 1	TCR-T + Cyclophosphamide + Fludarabine	NCT02869217	2016	Recruiting
	Phase 1	TCR-T + + Fludarabine Cyclophosphamide	NCT02366546	2015	Active but not recruiting
	Phase 1	TCR-T + + Fludarabine Cyclophosphamide	NCT02096614	2017	Unknown
	Phase 1	TCR-T + Radiotherapy	NCT03132922	2017	Recruiting
	Phase 1	TCR-T + Trastuzumab	NCT03680560	2018	Suspended by the sponsor
Peptide vaccine					
	Phase 1	+ Chemotherapy	NCT00632333	2011	Unknown
	Phase 2	+ Toll-like receptor 9 agonist	NCT00669292	2010	Unknown
	Phase 2	+ Granulocyte-macrophage colony stimulating factor	NCT00012246	2013	Terminated without any published results

Table I. Continued.

Immuno-therapeutic option	Stage of development	Combined with	ClinicalTrials gov.identifier	Start time	Current status
Oncolytic virus					
Oncolytic measles virus	Phase 1	+ 5-Fluorocytosine + anti-PD-1 checkpoint inhibitor	NCT04195373	2020	Withdrawn
Oncolytic adenovirus	Phase 1	CAdVEC combined with CAR-T	NCT03740256	2018	Recruiting
	Phase 1	Telomelysin + radiotherapy	NCT03213054	2017	Recruiting
	Phase 2	Telomelysin + Pembrolizumab	NCT03921021	2019	Recruiting

ClinicalTrials.gov was accessed in November, 2020.

is expressed, immunological tolerance exists and only in the case of a PD-1$^+$ tumour microenvironment containing CD4$^+$ T-cells an adoptive immune resistance is present that is most likely to respond to immune checkpoint inhibitor therapy (79).

5. Adoptive T-cell therapy

Adoptive T-cell therapy is a personalized approach of immunotherapy. T-cells are collected from the tumour or peripheral blood of a patient and the isolated T-cells are stimulated *in vitro* with IL-2. After this *ex-vivo* expansion, the cancer patient receives his own autologous immune cells as an infusion (80). In addition, T-cells can be also genetically modified after collection from the patient either by introducing chimeric antigen receptor (CAR T-cells) or transducing antigen-specific TCR cells (TCR T-cells). In all cases, the expanded or modified T-cells exert an improved tumour-specific immunity (81-83). In several trials, a regression of tumours has been demonstrated following persistent adoptive T-cell therapy (84,85). In a first clinical trial based on adoptive T-cell therapy for patients with recurrent or advanced oesophageal cancer, the patients received (on a fortnight basis) activated T-cells administered into primary tumours or metastatic lymph nodes; this therapy was found to be safe and in one third of the patients, a significant tumour regression was observed (86). In another study, based on TCR T-cells, oesophageal cancer patients with minimal tumours survived >27 months; nevertheless, after 2 months of treatment, several patients exhibited tumour progression even if the autologous T-cells persist for a long period of time; therefore, TCR T-cell therapy appears to have a benefit only for oesophageal cancer patients with minimal lesions (87).

6. Peptide vaccine

Peptide vaccines are therapeutic cancer vaccines which aim to increase immunogenic cancer-specific antigens, leading to the activation of cancer antigen-specific T-cells *in vivo* (59,76,88). For the successful use of peptide vaccines, the characterization of tumour-specific T-cells and the use of immunogenic tumour-associated antigens are a prerequisite (89). As tumour-associated antigens, either recombinant short peptides, whole-cell tumour lysates or full-length proteins can be used (90,91). The length of the used peptide has at least in part an influence on the efficiency of the immune response (92). It has been well-established that short peptides composed of 8-11 amino acids induce major histocompatibility complex (MHC) class-I-restricted antigen-specific CD8$^+$ T-cell reaction via direct binding to human leukocyte antigen (HLA)-I molecules (93). By contrast, longer peptides (25-50 amino acids) are usually presented by MHC class-I and class-II molecules on antigen-presenting cells to CD8$^+$ or CD4$^+$ T-cell, respectively (94). This results in a broader and longer lasting immune response by generating cytotoxic T-lymphocytes as well as long-living memory CD8$^+$ T-cells (95).

In a modified approach, dendritic cells isolated from the peripheral blood of a cancer patient are presented to tumour-associated antigens *ex vivo* and after loading with the antigens the dendritic cells, are re-injected into patients (91,96). This strategy was evaluated in a pre-clinical study as possible novel treatment option for oesophageal tumours (97). Dendritic cells from oesophageal cancer patients have been pulsed with Wilms' tumour 1 peptide *ex vivo* and used as a vaccine. The patients were treated in parallel with the chemotherapeutic agent, picibanil. In this exploratory study, 15 patients were included; the median progression-free survival and overall survival were 4.1 and 7.0 months, respectively. This treatment was well-tolerated and no severe adverse events related to the vaccinations were observed (97). Based on this promising result, a phase II clinical trial is in preparation.

Even with the first-generation of peptide vaccines which have been based on highly expressed non-mutant tumour-associated antigens of tumour cells [such as melanoma antigen gene (MAGE) and New York oesophageal squamous cell carcinoma-1 (NY-ESO-1) proteins] an immune response was induced and led to clinical positive effect (98-100). The advantage of these peptides is that they are only expressed in male

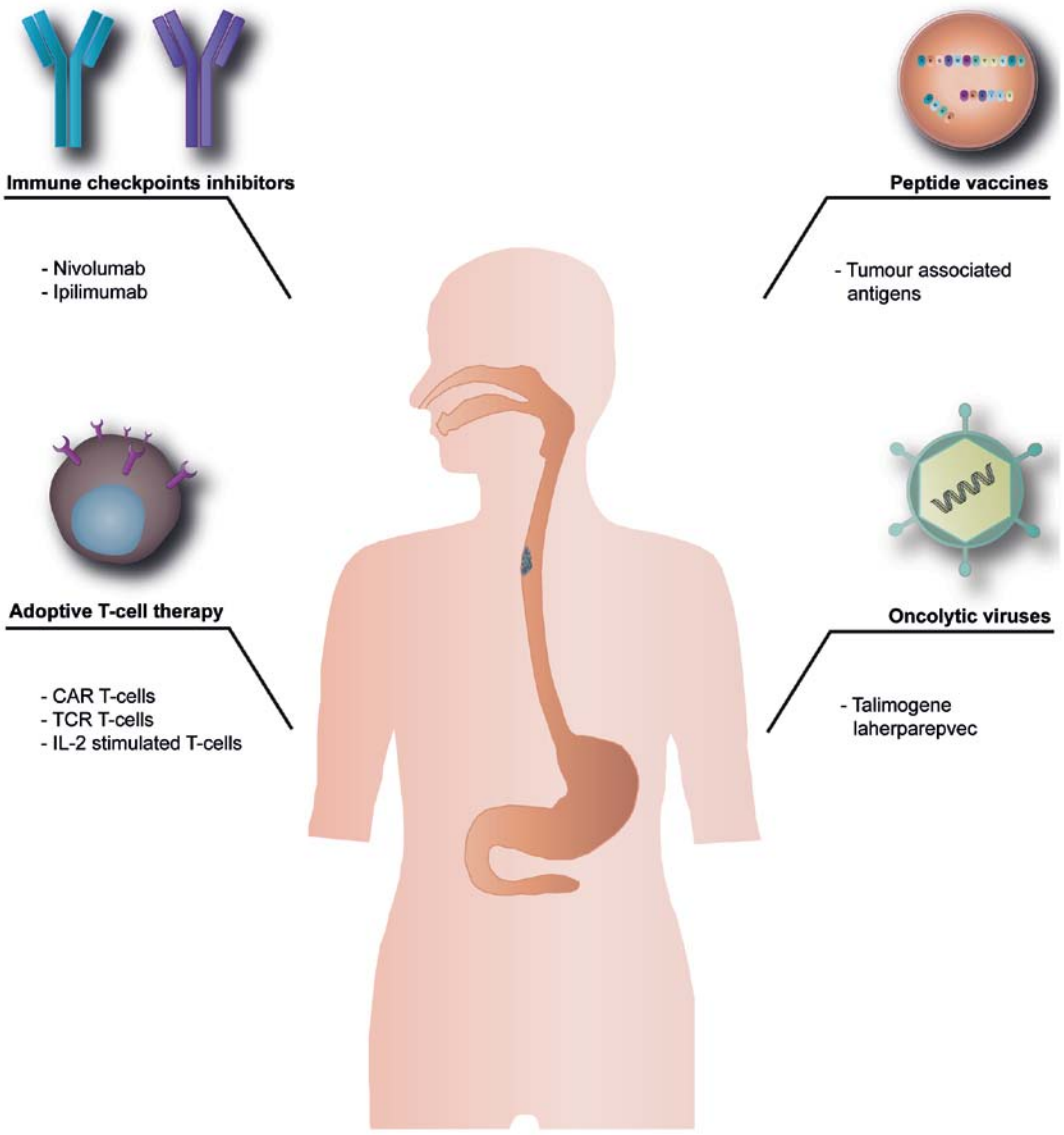

Figure 1. Immunotherapy approaches in oesophageal cancer. Illustration of the different immunotherapy approaches that have been studied thus far in the context of oesophageal cancer. CAR, chimeric antigen receptor; TCR, T-cell receptor; IL-2, interleukin 2.

germ-line cells and placenta under physiological conditions; however, a number of tumours, among these oesophageal cancer, express these proteins as well. Therefore, they represent very promising targets for cancer immunotherapy (101-103).

The second-generation of peptide vaccines is an effort for a more personalized medicine with the aim of targeting mutated antigens that are patient-specific. In this approach, mutations which have been accumulated during tumour development are the basis for the vaccine generation (104). In the context of oesophageal cancer, a large number of genetic mutations are present which result in specific neo-antigens (105). The main challenge is to identify mutated epitopes derived from tumour neo-antigens for developing a patient-specific vaccine (106,107). The vaccine peptides are patient-specific and they differ completely among patients. Therefore, batch production will not be possible and it will never become a conventional drug (104). The advantage is that neo-antigen vaccines result in a potent T-cell response and induce a new population of specific T-cells in cancer patients that are able to kill cancer cells without damaging healthy tissues (104,108,109). Furthermore, pre-clinical trials are forthcoming with an aim to induce the T-cell response by ribonucleic acid (RNA)-based vaccine coding for multiple neo-epitopes (110). Another novel strategy combines the use of long-peptide vaccines with checkpoint inhibitor administration (111). The aim in both cases, is to increase the repertoire of $CD8^+$ and $CD4^+$ T-cell directed against the tumour. These personalized approaches have the potential to offer novel therapeutic options with high specificity and low toxicity for cancer patients who are resistant to current therapies.

Peptide vaccines have been used in several clinical trials in patients with oesophageal squamous carcinoma. Different peptides have been administered simultaneously to patients, which resulted in a significant induced $CD8^+$ T-cell response. Clinical benefit, as well as an increased overall survival was observed in the majority of patients (112,113). Peptide vaccinations can be combined with other therapeutic options in patients with oesophageal tumours. One example is the use of a peptide vaccine to suppress the recurrence of oesophageal cancer following curative resection. In a previous study, the

5-year relapse-free survival of oesophageal cancer patients was 44.6% in patients that received the vaccination compared to the ones that did not receive the vaccination (31.6% relapse-free survival) (114). Of special interest is the peptide vaccine, S-588410, which is composed of 5 HLA-A*2402-restricted epitope peptides derived from the onco-antigens, DEPDC1, MPHOSPH1, URLC10, CDCA1 and KOC1. All these antigens are up-regulated in the context of oesophageal cancer (115,116). In previous studies, it was proven that each of these 5 peptides has the capacity to induce a peptide-specific activation of $CD8^+$ T-cells in different tumours, among these oesophageal cancer (112,113,117,118). In an exploratory study based on 15 patients with oesophageal tumours, an increased immune response in tumour tissue was observed following vaccination with S-588410. Following a median of 5 injections of S-588410, peptide-specific $CD8^+$ T-cells for all peptides included in this vaccination were induced in all patients. The number of functional T-lymphocytes ($CD8^+$ and $CD4^+$ T-cells) was found to be increased in blood, as well as in tumour biopsies. In parallel, a higher PD-L1 expression in the tumour microenvironment was observed (115). Most probably, the increased PD-L1 expression was related to interferon (IFN)-γ produced by infiltrated $CD8^+$ T-cells into the tumour area. The accumulation of effective T-cells and IFN-γ production in the tumour microenvironment most probably favour the change from an immune 'desert' into an immune-inflamed tumour microenvironment (93). It is tempting to speculate about the therapeutic potential of combining peptide vaccines, such as S-588410 with immune-checkpoint inhibitors in patients with oesophageal cancer (54,79).

7. Oncolytic viruses

Oncolytic virus therapy is still in its infancy, but it has already proven its potential. In general, oncolytic viruses infect and replicate selectively in tumour cells and induce tumour cell lysis (119,120). Talimogene laherparepvec is the first FDA-approved oncolytic viral therapy for the treatment of patients with advanced melanoma (121). Recently, the efficacy of a telomerase-specific oncolytic virus (telomelysin OBP-301) in combination with radiotherapy was investigated in a Phase I/II study for the treatment of elderly patients with oesophageal squamous cell carcinoma. According to the first results, this viral therapy was well-tolerated and demonstrated efficient tumour regression (122,123). Based on this success, several other clinical trials with various oncolytic viruses for the treatment of patients with oesophageal cancer are ongoing (Table I).

8. Conclusion and perspectives

In oesophageal cancer, as in most other tumour diseases, the therapeutic options are limited and therapeutic success is only achieved for a short period of time before resistance appears. Therefore, novel therapeutic options, such as the addition of immunotherapy to the treatment of tumours are an urgent need. Albeit some success of immunotherapy in oesophageal cancer treatment and the approval of pembrolizumab and nivolumab by the FDA, it is noteworthy to mention that immunotherapy is often associated with severe toxic side-effects; the most frequent ones are cutaneous, gastrointestinal, endocrine and hepatic toxicity. Therefore, a careful monitoring and follow-up of patients under immunotherapy is required and if necessary, the patient must receive effective measures to manage the side-effects. An advantage for patients with oesophageal cancer could be a combination of immunotherapy with surgery, chemotherapy and radiotherapy. Recently, the advantage from radiotherapy in parallel with immune checkpoint inhibitor treatment was already demonstrated (124).

A prerequisite for improving the success and efficiency of immunotherapy is the knowledge about robust biomarkers predicting clinical benefit before treatment and enabling stratification of oesophageal cancer patients in such a manner that the best possible immunotherapy can be applied to each patient. One possibility could be the multiplexed immunohistochemical staining of adaptive immune (CD3, CD4, CD8 and CD45RO) and immune checkpoint biomarkers [inducible T-cell costimulatory molecule (ICOS), indoleamine-2,3-dioxygenase-1 (IDO-1), PD-L1 and PD-1] in combination with digital pathology quantitation (125). Furthermore, it is well-established that immunotherapies are resulting in an increased tumour burden and/or emergence of new tumour lesions in the short-term. Therefore, the currently used evaluation system for therapeutically success is most probably not applicable for immunotherapies; thus, it may be prudent to consider a different system for this novel type of therapy.

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Authors' contributions

JCH, AL and NV were involved in the conceptualization of the study. JCH, MG, MR and AL were involved in the writing and preparation of the original draft. JCH, NV, MBM and AFO were involved in the writing, reviewing and editing of the manuscript. All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

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Competing interests

NV received speaker honorarium from the companies, Bayer, Eli-Lilly, Pfizer and Merck. The funders had no role in the design of the study; in the collection, analyses, or interpretation

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