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Chapter 2

Immunotherapy for Esophageal Cancer

Tian Wang and Yi Zhang

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http://dx.doi.org/10.5772/intechopen.78644

Abstract

As the third most common cancer of the gastrointestinal tract, esophageal cancer has a rather worse prognosis treated with current therapy strategies, and a poor 5-year survival rate lower than 15%. Recent years, emerging immunotherapy has showed a gratifying effect in treating other solid tumors which illuminates its usage for esophageal cancers. Immunotherapy for esophageal cancer basically includes adoptive-cell-therapy-based, antibody-based and vaccine-based therapies, and all of which have shown preliminary favorable results in treating esophageal cancer. However, due to the rather lower mutation rate and a tough microenvironment inside the cancer, promising immunotherapies like immune checkpoint blockade drugs, gene-modified-T cell therapies are hindered by the immunosuppressive factors from microenvironment. Future endeavors will be focusing on targeting immunosuppressive factors, combining immunotherapies with classical treatments to create a satisfying effect.

Keywords: esophageal cancer, tumor microenvironment, immunotherapy, immune checkpoint blockade drugs, CAR-T cell therapy

1. Introduction

Esophageal cancer is one of the leading culprits of mortality worldwide and is responsible for a total number of 746,000 [1] new cases and 439,000 [2] deaths every year. A large number of these cancers are diagnosed at an advanced stage and the metastasis causes bad outcomes. Endoscopic surgery, cytotoxic chemotherapy and radiotherapy remain to be the active and essential clinical treatment but only provide modest benefits, with median overall survival (OS) only in the range of 8–10 months [3]. After nearly 20 years of fast development, immunotherapy turned to be a promising method for treating esophageal cancer. Recent advances have brought some therapeutic regimens to be approved by Food and Drug Administration (FDA) then coming to clinical practice in first- and second-line settings (**Table 1**). The future

Treatment	Cancer type	Treating method	Phase	Study number
ACT				
	EC	CIK	II	NCT02490735
	Multiple cancer types (including EC)	CTL	I	NCT00004178
	EC	NY-ESO-1-TCR T cells	II	NCT01795976
Tumor vaccine				
Cell vaccine	Multiple cancer types (including EC)	Tumor cell vaccine	I	NCT01258868
	Multiple cancer types (including EC)	H1299 lysate vaccine	I/II	NCT02054104
	Multiple cancer types (including EC)	Allogeneic tumor vaccine	I	NCT01143545
Peptide vaccine	EC	IMF-001	I	NCT01003808
	EC	LY6K, VEGFR1, VEGFR2	I	NCT00561275
	EC	URLC10, TTK, KOC1, VEGFR1, VEGFR2, cisplatin, fluorouracil	I	NCT00632333
	EC	URLC10	I	NCT00753844
	EC, GC	G17DT, cisplatin, fluorouracil	III	NCT00020787
Immune checkpoints therapies	EC + GEJC + GC	Nivolumab/placebo	III	NCT02743494
	EC + GEJC Siewert I	Pembrolizumab vs. investigator's choice	III	NCT02564263
	EC	Pembrolizumab + brachytherapy	I	NCT02642809
	EC	Nivolumab vs. paclitaxel/docetaxel	III	NCT02569242
	EC + GEJC Siewert I	Pembrolizumab	II	NCT02971956
	EC + GC	Pembrolizumab + trastuzumab	II	NCT02318901
	EC	Durvalumab (anti-PD-L1) + chemoradiotherapy	I/II	NCT02735239
	Solid tumors (including ESCC)	LAG525 (anti-LAG3) + PD001 (anti-PD-1)	I/II	NCT02460224
	Solid tumors (including ESCC)	Nivolumab + ipilimumab	I	NCT02834013

ACT: adoptive cell therapy; CIK: cytokine-induced killer cells; CTL: cytotoxic T-cells; VEGFR: vascular endothelial growth factor receptors; PD: programmed cell death receptor; EC: esophageal cancer; ESCC: esophageal squamous cell carcinoma; GEJC: gastroesophageal junction carcinoma.

Table 1. Recent or completed clinical trials of potential therapeutic approaches for immunotherapy of esophageal cancer.

of immunotherapy is promising in treating solid tumors, yet still there is a desperate need for more effective and less toxic treatment options for patients with advanced esophageal cancer.

In recent years, emergence of immunotherapy towards cancer has improved the management of several malignancies dramatically, especially melanoma, renal cell carcinoma, non-small cell lung cancer and so on, shedding light on its usage on esophageal cancer as well. Identification of more suppressive factors in tumor microenvironment and insights into the biology of T cell functioning yielded a large number of diverse and novel anti-tumor regimens. Global efforts continue to explore how and when to integrate these agents in treatment of esophageal cancer. Immune checkpoint inhibition through antibodies that block cytotoxic T-lymphocyte antigen 4 (CTLA-4) and programmed cell death protein 1 (PD-1) has led to meaningful improvements in survival [4]. Vaccine adjuvant administration has also reduced tumor recurrence to some degree. CAR-T (chimeric antigen receptor T-cell) immunotherapy is a typical example of recent advances in synthetic biology, genome engineering, and cell manufacturing that has made it possible to develop highly specific and potent tumor-reactive T cells and offer many opportunities for experimentation and broader clinical translation. Combined regimens of immunotherapy with other classical esophageal treating procedures will illuminate the future of clinical oncotherapy. Recent achievements, which are illustrated below, including finished or ongoing clinical trials and laboratory findings of immunotherapy for esophageal cancer, bringing optimism for meaningful changes in treatment algorithms and for better outcomes in this fatal disease.

2. Immunotherapy regimens for esophageal cancer

2.1. History of immunotherapy

A few decades ago, several rare clinical regression of advanced cancer in response to immune stimulation aroused interests in the area of cancer treatment. A passionate handful of immunologists, oncologists and surgeons carried out forward-looking researches into the relations between tumor progress and body immune system. Their respectable efforts turned the seemingly insignificant clinical phenomenon into reproducible concrete success by revealing the hidden mechanisms of tumor response.

At the earliest period of cancer immunotherapies, the exact mechanism remained unknown and the anti-tumor growth effect was limited yet providing impetus for in-depth study. The first effective immunotherapies aiming at directly modulating cell function using well-characterized recombinant cytokines including interleukin-2 (IL-2) and interferon alpha (IFNα), yet the safety was lowered associated with substantial toxicity. Other cytokines, including IFNγ, IL-4, IL-7, IL-10, IL-12, IL-15, IL-18, IL-25, etc., failed to provide substantive benefit. The following breakthrough was the emerging conception of monoclonal antibodies (mAbs) targeting tumor cell surface receptor proteins (human epidermal growth factor receptor 2 (HER2)/Neu, epidermal growth factor receptor (EGFR), etc.) and were integrated into cancer care. Vaccination therapies using the modified peptide, whole tumor, recombinant proteins, dendritic cells (DCs), and adjuvants were only modestly successful. With

researching deeper into the cross-talk happening in the tumor microenvironment between tumor cells and immune cells, the modern era of immunotherapy launched with the extraordinary novel efficacy (and toxicities) of mAbs targeting immune checkpoints in patients with various cancer types in order to decrease the suppressive potential that would prevent immune cells from functioning. Current alternative approach to overcoming the suppressive tumor microenvironment was the administration of genetically modified autologous T cells targeting specific cancer-related antigens [5]. This could be done in the form of T cells in which modified specific T cell receptors (TCR) were inserted for a shared tumor antigen or a tumor-specific neoantigen accompanied by co-transduction of stimulatory cytokines such as IL-12 or could co-administrate with PD-1 pathway blockers to sustain the capability before being expanded in large numbers *in vitro* following lymphodepleting chemotherapy. These approaches have produced dramatic responses in a few patients with a variety of individual tumor types, especially hematological malignancies [6]. Further improvement of adoptive cell transfer therapy came from the modified T cells expressing chimeric antigen receptors (CAR) targeting tumor-specific binding domains. After several generations of amelioration, CAR-T therapy showed extraordinary effect in treating hematological malignancies yet treatment of patients with solid tumors using CAR-T cells had been less fruitful and had troubled clinicians much through the off-tumor/on-target toxicity. Given the virtually limitless possibility of *ex vivo* engineering of T cells, many of these potential hurdles will likely be resolved by the additional modification of T cell products prior to treatment.

Immunotherapy showed great potential in the treatment of esophageal cancer. Modern treatments of patients with esophageal cancer integrated immunotherapy with conventional surgical, chemotherapeutic, and radiation oncologic strategies. In spite of the seemingly gratifying results, the 5-year survival rate of patients with esophageal cancer in the middle and late stages is still lower than 15%, the 5-year survival rate of patients with locally advanced surgery alone is only 20–25% [7]. Postoperative chemotherapy or neoadjuvant chemoradiation only turns the 5-year survival rate up to only 30–35% [8]. One of the causes to blame of the rather poor prognosis of esophageal cancer is the rapid disease progression. More than 50% of patients already have visible metastases at the time of diagnosis [7]. Therefore, further investigation of esophageal cancer microenvironment and its impact on disease progression will lay a solid theoretical foundation for early diagnosis and treatment improvement of esophageal cancer.

In this chapter, we tried to converge concepts relevant to the complicated relationship between the host and the neoplastic tissue.

2.2. Immune microenvironment and molecular correlations in esophageal cancer

As early as 100 years ago, Paget [9] had put forward the hypothesis of "seeds and soil", which laid the foundation for the concept of tumor microenvironment. Numerous data indicate that many immune-related cells, factors and immune-related signaling pathways in the tumor microenvironment play an important role in the occurrence, metastasis, recurrence, angiogenesis, and drug resistance of tumors. The in-depth study of the tumor immune microenvironment is to search for molecular pathogenesis and new therapeutic models of esophageal cancer.

Tumor microenvironment is constituted by various immune cells, endothelial cells, adipocytes, paravascular cells, nerve cells, fibroblasts, and extracellular matrix components around the cancer cells [10]. Some stromal cells in the tumor microenvironment have immunosuppressive potential to inhibit the function of immune effector cells and promote tumor progression. Thus inhibiting cancer cell apoptosis and promoting mechanisms such as proliferation, angiogenesis, drug resistance, and immune escape to promote tumorigenesis [11]. Chemokines CCL17 and CCL22 secreted by tumor cells or tumor-associated macrophages (TAMs) recruit CCR4+ regulatory T cells (Treg), which then inhibit immune effector cells by contacting directly or secretion of cytokines (IL-10 and IL-35). Th17 cells can be transformed into Treg cells stimulated by IL-6 and transforming growth factor-β (TGF-β). Inflammation and tumor-derived factors stimulate the activation of myeloid-derived suppressor cells (MDSCs). Activated MDSCs can directly inhibit the expression of CD8 + T cells. Activation and induction of Treg cells and other mechanisms contribute to immune escape of cancer cells. TAM cells and CAF cells promote the growth, invasion, metastasis, and angiogenesis of tumor cells through the secretion of cytokines, chemokines, and various growth factors. In addition, tumor cells and TAM cells can express programmed cell death ligand (PD)-L1/2 inhibiting T cell activation after binding to PD-1.

MDSCs have been shown to play an important role in promoting tumor immune escape, activating CAF cells and angiogenesis [12]. The presence of proinflammatory cytokines such as IL-1, IL-6 and prostaglandins in esophageal cancer microenvironment can activate MDSC [13]. MDSC inhibits activation of T cells by direct inhibition [14], cytotoxicity of natural killer cells (NK) [15], depletion of arginine and cysteine, induction of Treg cells, etc. to achieve immune escape [16]. Another group of immunosuppressive cells that exert similar functions are Treg cells. Under physiological conditions, Treg cells can regulate the activation and proliferation of T cells, B cells, and cytotoxicity of NK cells. But in tumor microenvironment, Treg cells can promote the occurrence and progression of tumor cells by secreting immunosuppressive related factors, secreting tumor-associated antigens (TAAs), and suppressing the cellular adverse reactions of immune effector cells and the release of granzymes [17]. Studies have shown that tumor cells and TAMs can recruit CCR4+ Treg cells to tumor sites by secreting CCL17 and CCL22 and other chemokines [18]. Treg cells are highly aggregated in tumor cites, promoting tumor invasion and metastasis, and are associated with disease severity, survival after chemotherapy, and prognosis [17]. Additionally, Th17 cells can secrete IL-17 and IL-22 and activate STAT3 related signaling pathways to promote angiogenesis and tumor growth [23]. However, the role of Th17 cells is still controversial. What factors affect the function of Th17 have not yet been well defined [18]. Therefore, we still have to know more about Th17 cells in esophageal cancer to explore potential therapeutic targets.

The tumorigenic mechanisms of TAMs are varied. Phenotype spectrum of macrophage ranges from M1 to M2: M1 macrophages represent the classical activated macrophages, with functions of cytokines secretion, antigen presentation, resistance to infection and anti-tumor ability, etc., while M2 macrophages secrete type II cytokines and induce activation of COX2/prostaglandin E and other mechanisms that cause tumorigenesis [19]. The presence of cancer associated fibroblasts (CAFs) in patients with esophageal cancer is associated with microvessel density, and can also promote tumor progression and metastasis through epithelial mesenchymal transition (EMT). CAFs are also associated with 3-year survival rates and disease recurrence after radiotherapy and chemotherapy [20].

PD-1 is a member of the CD28 superfamily and is an important immunosuppressive molecule that inhibits the activation of T cells after binding to its ligand PD-L1/PD-L2 [4]. Multiple experiments confirmed that PD-L1 and PD-L2 are highly expressed in esophageal cancer [21], in which PD-L1 expression is closely related to tumor invasion depth and poor prognosis, whereas PD-L2 expression is associated with decreased CD8+ T cell infiltration [21]. The increasing PD-L2 expression can promote the secretion of Th2 cytokines such as IL-4/IL-13 [22]. These pieces of evidence suggest that blockers targeting PD-1 are of great significance in the treatment of esophageal cancer [23].

In the early stages of esophageal cancer, TGF-β signaling suppresses tumor growth by downregulating the expression of Smad4 and c-Myc genes, while promoting growth and EMT in advanced esophageal cancer [24]. This "switching" effect is thought to be caused by the loss of adaptor proteins. For instance, an important adaptor protein, β2-spectrin, plays an important part in cell–cell interactions and maintenance of epithelial cell polarity. In esophageal adenocarcinoma, decreasing β2-spectrin in tumor cells results in increased expression of SOX9 and c-Myc, but it also reduces other TGF-β targets such as E-cadherin and cell cycle-regulated p21 and p27 genes [25]. In summary, these changes make TGF-β promote the progression and metastasis of the tumor by inducing EMT, especially in epithelial tumors like esophageal cancer.

In addition to growth factors, chemokines in the tumor microenvironment also play an important role in the development of tumors. Mainly, there is stromal cell derived factor-1 (CXCL12/SDF-1) secreted by fibroblasts [26], binding to its corresponding receptor CXCR4 or CXCR7 thus inducing tumor growth, promoting angiogenesis, stimulating tumor movement, invasion and metastasis [26]. SDF-1/CXCR4/CXCR7 axis is closely related to tumor invasion, metastasis and survival. However, the use of these separate components as indicators of prognostic analysis has yielded inconsistent results [27]. Nonetheless, CXCL12 has been shown to regulate the migration of CXCR4-positive tumor cells in esophageal adenocarcinoma *in vitro* and *in vivo*. Knockout of CXCR4 expression in KYSE-150 and TE-13 esophageal cancer cells by small interfering RNA can inhibit the proliferation, invasion and metastasis of tumor cells. Local CCL5 and CXCL10 in esophageal squamous cell carcinoma can recruit CD8+ T cells to the tumor site [28].

Further researches showed that remodeling body immune state by various means in esophageal cancer patients will be the main research direction of immunotherapy for esophageal cancer.

2.3. Checkpoint inhibitor regimens

Immune system has sophisticated regulatory mechanisms. Several checkpoints are involved to maintain the balance between effective immune-responses fighting against infection or cancer state yet won't activate excessively to prevent damaging healthy cells. Cytotoxic T lymphocyte antigen-4 (CTLA-4) and PD-1 are among the major inhibitory receptors expressed by Tregs which downregulate immune responses [29]. Inhibitory antibodies modulating these immune checkpoints have been most frequently used in immune-oncology trials in esophageal cancers.

PD-1/PD-L1 blockers have achieved encouraging clinical results in treating melanoma, lung cancer and other cancer types [30, 31]. The potential role of PD-1 blockers in treatment of esophageal cancer can be predicted by genomic map of the tumor immune microenvironment. The expression of PD-L1 on MDSCs isolated from esophageal cancer tissue was higher obviously, and PD-1 expression was detected in approximately 60% of the tumor infiltrating lymphocytes (TILs) from esophageal cancer tissues [32]. Therefore, inhibition of the PD-1/PD-L1 pathway for esophageal cancer treatment cannot be ignored.

Preliminary results of ongoing studies indicated that PD-1 blocker Pembrolizumab had acceptable safety in PD-L1 positive esophageal cancer patients. The objective response rate for mid-term analysis was approximately 30% with a sustained response period of up to 40 weeks [33]. These results laid the foundation for the continued completion of checkpoint inhibitor regimens pivotal study in esophageal cancer patients.

CTLA-4, also known as CD152, belongs to the immunoglobulin superfamily and serves as an immunological checkpoint. When activated CD4+ helper T cells express CTLA-4, this kind of cells sends inhibitory signals to T cells [34]. High CTLA-4 expressing CD4+ Treg cells block T cells by reducing IL-2 secretion and downregulating IL-2 receptor expression then retard T cells in G1 phase of the cell cycle [35]. Ipilimumab and tremelimumab have two fully humanized monoclonal anti-CTLA-4 antibodies that have already received FDA approval for the treatment of melanoma and mesothelioma [36, 37]. A survey of tremelimumab has been completed in phase II clinical trials for treating advanced gastric and esophageal cancer (n = 18). Although only a 5% response rate was observed, four patients were controlled and one patient was observed with partial remission (25.4 Mo) after treatment and continued for several months [38]. The results of ongoing clinical trials are expected to further highlight the clinical value of monoclonal anti-CTLA-4 antibodies in esophageal cancer.

2.4. Vaccine regimens

Tumor vaccine treatment involves the administration of TAAs into patients thus triggering specific anti-tumor immune responses. Rosenberg et al. [39, 40] conducted a comprehensive review of 1306 cancer vaccine usage studies conducted before 2004 and found that the overall target response rate was only 3.3%. The explanation may be that these immune cells have low affinity or are inhibited by endogenous factors like the checkpoints mentioned above.

For the treatment of esophageal cancer, some vaccine-based clinical trial reports have been published. A Phase I clinical trial of 10 patients with refractory stage III or IV esophageal squamous cell carcinoma treated with peptide vaccine found that 9 patients developed antigen-specific T cell immune response. One of the patients with liver metastases showed complete remission for 7 months, another had partial remission within all metastatic lung lesions, and 3 patients had progression-free survival lasting 2.5 months. The peptide vaccine used was derived from three HLA-A24-restricted cancer testis antigens (TTK protein kinase, lymphocyte antigen 6 complex locus K, and insulin-like growth factor-II mRNA binding protein 3) [41]. The multicenter, phase II clinical trial of the vaccine evaluated the OS, PFS, and immune response after vaccinations in patients with HLA-A*2402 positive and negative esophageal squamous cell carcinoma, and immune response was observed in HLA-A*2402

positive patients (n = 35), but there was no statistical difference in OS compared to HLA-A*2402-negative patients (n = 25) (4.6 mo vs. 2.6 mo, P > 0.05), yet there is a significant difference in PFS (P = 0.032) [42]. In a tumor vaccine trial hosted by Saito et al. [43] (n = 20), 4 patients with high levels of MAGE-A4 or MHC class I antigen in autologous tumor cells not only showed MAGE-A4 specific immune responses after vaccination, but compared with patients without using antibodies, their OS was also significantly prolonged. Wada et al. [44] used NY-ESO-1 as a cancer vaccine in 8 patients with esophageal cancer. The results showed that 7 patients had an immune response. Of the 6 patients evaluated for efficacy, 1 patient experienced partial remission, 2 patients continued to maintain progression-free status, and 2 patients had mixed clinical responses. Given the preliminary results of these peptide vaccines in clinical trials, safety inspections and the related researches combined with radiotherapy and chemotherapy are also being carried out gradually in treating multiple cancer types including esophageal cancer.

2.5. Adoptive cell therapy regimens

The concept of ACT (adoptive cell therapy) was first proposed by Dietrich et al. [45]. It refers to the treatment of utilizing autologous or allogeneic immune cells by infusing them back into the patients after being amplified *in vitro* by certain means. Currently effector cells can be divided into two categories: the first type is non-specific immune cells, including autologous lymphokine-activated killer cells (LAK), cytokine-induced killer cells (CIK) and NK cells. Cells are isolated from peripheral blood cells and are stimulated by lymphokines or cytokines; another type of effector cells is antigen-specific T cells, including TILs, cytotoxic T cells (CTL) and genetically engineered T cells including T cell receptor transferred T-cells (TCR-T) and CAR-T [46].

The first ACT trial in human improved the survival of patients with metastatic cancer by reintroduction of CIK and recombinant IL-2 to their body, which has been successfully applied to the treatment of refractory metastatic melanoma, and for other types of cancer such as glioma, renal cell carcinoma, non-small cell lung cancer, etc. The objective response rate varies from 20 to 72% [47], encouraging its further usage in esophageal cancer, too.

So far, ACT treatment of esophageal cancer has been evaluated in several clinical trials. In the first published study by Besser et al. [48] and Toh et al. [49], mononuclear cells were isolated from peripheral blood of esophageal squamous cell carcinoma patients and were given autologous tumor cell stimulation *in vitro*. Latter results showed that half of the patients had an objective response, and 36% of the subjects achieved complete remission or partial remission. CTL and TIL cells are now hot spots for carrying out immunotherapy for solid tumors as the mechanism of killing tumor is rather clear.

TCR-T cells transduce the α and β chains of the antigen-specific high-affinity TCR into T cells and express them on the cell surface, thereby effectively identifying and killing the tumor cells expressing the antigen. Currently, the most common TAAs found in esophageal cancer are cancer testis MAGE-A3/4 and NY-ESO-1. Several studies [50, 51] showed that the expression ratio of MAGE-A3 in esophageal cancer was about 90%, and the expression rate of NYESO-1 in esophageal cancer was up to 40–90%. A preliminary phase 1 clinical trial

of genetically engineered T cells was carried out by Kageyama et al. [52], TCR-T cells were readopted to patients with MAGE-A4-positive recurrent esophageal cancer, and administered the MAGE-A4 peptide vaccine subsequently, the level of TCR-T cells in the peripheral blood of 10 subjects was monitored for 5 months, and 5 of them were able to detect specific T cells continuously. Seven subjects appeared tumor progress after 2 months of treatment, but another 3 subjects survived more than 27 months.

CAR-T is another type of genetically engineered T cells. CAR-T was obtained by translocating chimeric antigen receptor such as CARs into T cells. Gross et al. [53] successfully constructed the structure of CARs into T cells for the first time to exert their specific killing function. Up to now, tens of clinical trial data of CAR-T treatment on malignant hematological malignancies have been published. The Novartis' tisagenlecleucel, a synthetic bioimmune product of anti-CD19 CAR-T cells has been approved by FDA on treating relapsed/refractory B-cell acute lymphoblastic leukemia (B-ALL) in 2017 [54]. The early generation of CAR-T therapy for solid tumors did not appear ideal outcomes during clinical use [55]. The reasonable explanation might be lack of unique TAAs in solid tumors, less efficiency and persistency of T cell homing to tumor sites, and the intratumoral immunosuppressive environment strongly inhibited CAR-T cell function. Clinical trials targeting solid tumors using second or third-generation CAR techniques had been limited, but some of the more significant clinical trials are carrying out good results. Patients with metastatic or recurrent HER2-positive medulloblastoma treated with HER2-CAR-T had came out with stable conditions [56]. Feng et al. [57] showed the results of a clinical trial of an EGFR-CAR-T treatment for EGFR-positive relapsed/refractory NSCLC patients revealed partial remission in 2 of the 11 patients involved in the evaluation and 5 patients had a stable condition ranging from 2 to 8 months. There were no obvious adverse reactions in the entire clinical trial. In the treatment of esophageal cancer, no CAR-T therapy-related studies have been conducted yet. However, anti-tumor targets are now erupting in esophageal cancer. Points such as HER2 can also provide references for future steps in the development of research.

3. Combined treatment

Currently, comprehensive therapy shed light on tumor treatment to a large degree. Clinical practice has proven that it is difficult to achieve the best results with any single treatment method. Therefore, the principle of treatment for most tumors depends on comprehensive treatment. Recent research results showed that the combined usage of immunotherapy and chemotherapy in a variety of cancer treatment achieved better results than a single therapy, it can not only reverse the immunosuppression effects caused by the late stage of the tumor, increase the cross-presentation of tumor antigens, promote the proliferation of killer T cells and make it more easy to kill tumor cells, but can also reduce the incidence of adverse reactions from chemotherapy and reduce drug resistance of tumor cells to some extent. Combination of chemotherapy and immunotherapy has been a common available method in some cancer types. Neoadjuvant chemotherapy regimens together with HER2-targeted therapy achieved pathologic complete response in relapse-free survival among patients with breast cancer [58].

New treatment targeting specific mutant genes illustrated clinical success in a phase II study of metastatic melanoma combined with interleukin-2 aldesleukin and BRAF inhibitor vemurafenib [59]. The effectiveness of combined therapy can even cover the metastatic areas, in melanoma brain metastases, complete intracranial response was observed after using dual checkpoint-inhibition of talimogene laherparepvec (T-Vec), pembrolizumab and whole brain radiotherapy. Additionally, immunotherapy also showed potential augmented effect plus cryotherapy [60], focused ultrasound therapy [61] and photothermal therapy [62], etc. Tumor vaccine also took another leap when combined with other immunotherapies, clinical results showed that nivolumab in combination with talimogene laherparepvec (T-Vec) in resected melanoma carried out better outcomes [63]. The clinical value of immunotherapy and radiotherapy showed even more outstanding clinical effects, anti-tumor immune response was enhanced by anti-PD-1 immunotherapy in recurrent nasopharyngeal carcinoma, showing a bright prospect of further combination [64]. In treating esophageal cancer, there are also many creative comprehensive treatment methods that are constantly developing. In patients with drug-resistant esophageal cancer, a phase Ib/II study of low-dose decitabine-primed chemoimmunotherapy showed undeniable safety and efficacy [65]. A combination therapy of multi-peptide vaccine with chemoradiation therapy performed satisfying safety thus can be an effective treatment for patients with unresectable ESCC [66]. Novel multitarget tyrosine kinase inhibitor anlotinib in a third-line treatment of refractory advanced non-small-cell lung cancer (RA-NSCLC) provided significant PFS benefits compared with placebo, and accompanied with acceptable toxicity [67]. Though has a guaranteed future, there need to be more rational and effective trials of combinations to be excavated in the field of treating esophageal cancer together with other solid tumors.

4. Conclusions and future directions

The success of immunotherapy in some tumors came from years of research deep into the immune system and tumor itself and brought hope of healing cancer. It is worth mentioning that several immune checkpoint blockers have been or are being approved by the FDA, and it is expected that the next step will be to accelerate the pace of application of single drug or other treatment modes in combination within clinical usage. However, opportunities and challenges coexist, and there are still some key questions that have not been answered in immunotherapy. First of all, many targeted cancer drugs that treat cancer need to be explored to determine the biological dose that achieves the greatest clinical benefit with minimal toxicity. Second, given that most of the current immunotherapy is mainly to activate anti-tumor effects by activating the immune system, this kind of treatment requires patient to have some degree of immunity before receiving the initial immunotherapy. Therefore, it is imperative to fully evaluate patient's immune status and find biomarkers that predict the effectiveness of immunotherapy. In addition, there is abundant evidence that exposure to radiation and chemotherapy drugs may affect the rate of DNA mutations in tumor cells, prompting the formation of some new antigens. As the current immunotherapy is always combined with radiotherapy and chemotherapy, determining the proper dose for each regimen is the prerequisite for maximum benefit of combined therapy.

Immunotherapy has a broad application prospect in the treatment of malignant tumors. The high frequency of esophageal cancer mutations and the effective results of immunotherapy highlighted in other gastrointestinal cancers provide strong evidence for the study of esophageal cancer immunotherapy. Treatment strategies combined with existing or new treatment modes will be the direction of future esophageal cancer treatment.

Author details

Tian Wang and Yi Zhang*

*Address all correspondence to: yizhang@zzu.edu.cn

Biotherapy Center, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China

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