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Programmed cell death 1 as prognostic marker and therapeutic target in upper gastrointestinal cancers

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Negar Khoshghamat, Niloufar Jafari, Mohammad-Hossein khajavi, Saeed Sahebdel, have gathered study data and written the manuscript. Ghazaleh Khalili-Tanha, Mehrdad Moetamani-Ahmadi, Nima Khalili-Tanha, Saman Soleimanpour, Majid Khazaei, Seyed Mahdi Hassanian, Gordon A. Ferns, Amir Avan have provided critical revision of final manuscript. Saman Soleimanpour, Majid Khazaei, Seyed Mahdi Hassanian, Gordon A. Ferns, Amir Avan has contributed to the study design and approved the final version of the manuscript. All the authors read and approved the final version of the manuscript.

ABSTRACT

Gastrointestinal (GIs) cancers are among the most common causes of cancer related death, and hence the importance for the identification of novel prognostic/predictive biomarkers for detection of patients at an early stage, and for using these to identify novel targeted therapies to improve the efficacy of existing chemotherapeutic regimens. Programmed cell death 1 has been reported as a potential target in several malignancies, and targeting agents are being developed, some already approved by FDA, such as: pembrolizumab, Atezolizumab, Nivolumab. Pembrolizumab that have been approved for the treatment of metastatic non-small cell lung cancer. Here we provide an overview of the mechanism of action PD-1/PD-L1, prognostic value and current progress in clinical trials using PD-1/PD-L1 inhibitors, and the resistant mechanisms at underlie the inhibitory effect of these agents in the treatment of gastrointestinal cancers.

Key words: Upper gastrointestinal cancers; Programmed cell death 1; Immune therapy; Biomarker; Resistance

1.1.INTRODUCTION

Gastrointestinal cancers (GICs) are a common cause of cancer related morbidity and mortality worldwide [1,2]. Upper GICs comprise esophageal, gastroesophageal junction (GEJ) and gastric cancers, with overall five-year survival rates of approximately 15-25%, 2-12% and 31%, respectively [3–9]. Depending on the type of cancer, there are a variety of treatments used for GICs include chemotherapy, surgery, radiation therapy, targeted therapy and also immune checkpoint therapy.

In recent years, immune therapy, that targets regulatory pathways in T cells to increase the immune response to the tumour, has made considerable advances. This treatment has produced long-term remission in a large number of patients who have not shown any response to other treatments [10,11]. Immune checkpoint therapy may also provide worthwhile information on identifying other pathways that could be targeted through combination therapies to allow survival benefits for patients [11]. Although immunotherapy is not yet approved as a treatment, it is apparent that many GICs are sensitive to this type of therapy [10].

Inhibiting PD-L1 and the PD-1 receptor appears to be a potent form of immunotherapy [12]. The binding of PD-L1 to PD-1 on T cells leads to T cell inefficiency that permits tumour cells to evade immune mechanisms [3]. There is also a need to promote non-invasive methods for the early detection of GICs [1]. Currently, there remains a need for predictive and prognostic biomarkers of GICs [1,13,14]. In spite of noticeable development in this field, few biomarkers with high enough sensitivity and specificity have been identified [1,14]. In this review, we

highlight the current status of PD-1/PD-L1 targeted therapies and also summarize its value as biomarker in upper GICs.

1.2. PD-1 signalling pathway

Cancer cells express different antigens from normal cells due to genetic and epigenetic abnormalities. The immune system of the host uses these antigens to identify cancerous cells [15], and then attempts to destroy the cancer cells before a tumour develops, a process known as immune surveillance. The balance between stimulatory and inhibitory molecules are of importance [16]. T cells are the main elements of the immune system that respond to tumour development. Tumour cells may evade the immune response and become clinically apparent, a process called immune escape. There are several mechanisms for immune response evasion, including secretion of soluble inhibitory factors (TGF- β , VEGF, IL-10, etc.), low expression rates of major histocompatibility complex (MHC) class 1, and increase in cell surface expression of immune checkpoint ligands [17]. The PD-1 gene encodes a type-I transmembrane protein, which is involved in immunological regulation in viral infection, autoimmunity including systemic lupus erythematosus and rheumatoid arthritis, and transplant immunity besides cancer immunology [18]. PD-L1 is also called B7 homolog 1 (B7-H1) or CD274. It is known that PD-1 restrains human T cell response, and engaged by ligand, PD-1 inhibits T cells activation through phosphatase activity, which blocks kinase signalling pathway [19]. PD-L1 was later identified as a binding and interacting partner of PD-1 [20]. Recently, PD-1, CTLA-4 receptors on T-cells, and PD-L1 are determined as clinical targets. PD-1, also noted as CD279, is an inhibitory receptor on cellular surface which suppresses immune response and causes tumour immune

escape during carcinogenesis [21]. Tumour cells express PD-L1 in response to particular cytokines, specifically, IFN- γ . Given that thymocyte-1 (Th-1) type helper CD4 cells, activated B cells, and natural killer cells produce IFN- γ , this mechanism shows that cancerous cells can detect an inflammatory immune microenvironment that potentially inhibits tumour formation [22]. Dendritic cells selectively express PD-L2, another PD-1 ligand, leading to inhibition of T cell receptor-mediated response through interactions with PD-1 [23]. PD-1: PD-L1/PD-L2 interaction blocks T cells activation at tumour location, whereas CTLA-4 inhibition blocks activation of the T cell in lymphoid tissue [19]. Unlike PD-L1, tumour cells do not usually over express PD-L2 [16]. These two ligands have been shown to cooperate with other ligands, as PD-L2 collaborates with CD80 and CTLA-4. Its Interactions can be controlled by immune checkpoints. These checkpoints appear be highly important regarding a variety of tumours, as long as they can cause the formation of exhausted T cell phenotype with deficiency in proliferation and cytolytic activity causing loss of cytokine secretion and finally deletion error. Tumour-infiltrating lymphocytes (TILs) express a large proportion of PD-1 regarding a variety of tumors[GF1] . A rate of 20-50% of human tumours can express PD-L1, which can help many types of cancers to evade immune response by PD-L1 over expression. For, PD-1 and its ligands interaction blockade can partially reduce tumour evasion and improve the response, and can be used as a potential therapeutic option in tumour inhibition [24].

2. EBV positive GICs

It is known that in some cases, gastric cancer occurs following EBV infection [25]. The monoclonal proliferation of the cells occurs via PD-L1 and PD-L2 overexpression pathways [26,27]. JAK2 amplification besides DNA hypermethylation and recurrent PIK3CA mutations are

some of the other means by which EBV-associated gastric cancer develops [27]. Infiltration of T-lymphocytes into the EBV-infected regions is unavoidable. These infiltrating T-cells release IFN- γ which initiates PD-1/PD-L1 pathway signals [28]. Therefore, immune checkpoint therapy via PD-1/PD-L1 pathway blockade may be a promising treatment of the mentioned gastric cancer [29,30].

3. Upper GICs drugs targeting PD-1 and PD-1R

There are several lines of evidence that suggest that inhibition of the PD-1/PD-L1 pathway may be a therapeutic option in UGI tumours. As discussed above; PD-L1 is expressed on APCs and cancer cells, while PD-1 genes are turned on in T-Cells. This mechanism suggests the potential efficacy of PD-1/PD-L1 blockade to induce cellular immunity and result in solid tumour shrinkage and improve the patient's condition in the presence of metastasis [31]. Much of the current literature pays particular attention to the potential effects of Pembrolizumab, SHR-1210, and Nivolumab alone, or in combination with other agents. The way that these therapies affect PD-1/PD-L1 is similar and the difference is based on their activities [32]. Additionally, there is a growing body of literature on the best therapeutic combination for these patients. Here we review published, and ongoing clinical trials that have been designed to explore the best therapy for GICs (table1, figure 1).

3.1. *Pembrolizumab (KEYTRUDA)*

Pembrolizumab is monoclonal human immunoglobulin G4- κ which targets PD-1. It has been shown to have a significant overall response rate (ORR) in various tumour therapies including advanced esophageal cancer, gastric cancer and some adenocarcinomas. In 2016 the FDA

approved Pembrolizumab in the treatment of head and neck cancer [33]. In the phase1 KEYNOTE-012 Study the data showed that the ORR was 22% and its promising antitumor activity on advanced solid tumours led to the subsequent following-up studies (NCT01848834). Merk sharp *et al.* have designed a phase 2 KEYNOTE-590 study on 133 metastatic gastric cancerous patients and the result showed an ORR of 16.4%. Ken Kato *et al.* designed a KEYNOTE-590 phase 3 trial to assess the efficacy and safety of pembrolizumab in combination with cisplatin plus 5-FU in 700 cases of locally advanced or metastatic esophageal carcinoma. The results of this ongoing trial show potential effects of Pembrolizumab in advanced/metastatic adenocarcinoma SCC patients (NCT03189719). Nanda R *et al.* designed a phase 1b study for gastric, triple-negative breast, urothelial, and head and neck cancers. Among the 27 patients who were evaluated for antitumor activity was 18.5% (NCT01848834). A phase II study is ongoing. (NCT02447003).

3.2 SHR-1210 (Camrelizumab)

SHR-1210 is an IgG4- kappa monoclonal antibody directed against human PD-1. There are several clinical trials in different phases of development that have been designed to determine the effect of SHR-1210 alone or with other therapies [34]. SHR-1210 binds to PD-1 with high affinity, leading to the inhibition of PD-1 signalling and results in a more favourable immune response through T-Cell induction [35].

In a phase 1 trial efficacy and safety of SHR-1210 in 30 patients with advanced gastric/GEJ cancer. 23.3% of them demonstrated an objective response. Mo H *et al.* also conducted a study on 36 patients with advanced solid tumours. In this trial SHR-1210 was administered

intravenously and the result showed promising antitumor activity, with seven partial responses (=19%).

Thirty two patients with advanced solid tumours received SHR-1210-activated MASCT (aMASCT) alone and 38 patients received aMASCT plus apatinib after standard treatment. SHR1210-aMASCT cells had a promising beneficial effect in patients with advanced solid tumours. Xu J et al. recently published the data of a phase 1a/b study in patients with advanced hepatocellular carcinoma, gastric, or GEJ cancer. They report that in a combination therapy, toxicity is manageable, and its application in these patients was beneficial ([NCT02942329](#)).

3.3 Nivolumab

Nivolumab is a FDA approved humanized monoclonal IgG4 for various malignancies including melanoma, head and neck squamous cell carcinoma, renal cell carcinoma, squamous non-small cell lung cancer [31,36]. Nivolumab has been found to boost T-Cell response. Conversely, no reportable effect on antibody-dependent cell-mediated cytotoxicity and memory T-Cells in in-vitro experiments has been reported [37].

Meindl-Beinker NM et al[GF2] . compared the effects of combined nivolumab/ipilimumab with standard treatment. This ongoing trial will recruit 75 patients ([NCT03416244](#)). Another open-label, multicenter, phase 2 trial designed to investigate the safety and efficacy of nivolumab in 65 patients with treatment-refractory esophageal cancer. Primarily findings of this ongoing trial showed promising nivolumab effect with a manageable safety profile (JapicCTI-No.142422).Patients with advanced gastric or GEJ cancer who showed resistance to chemotherapy participated in another phase 3 trial. ([NCT02267343](#))493 patients received

Nivolumab or placebo and the data analyzed. Median overall survival calculated 95% in the Nivolumab group. These findings indicate the beneficial effects of Nivolumab.

3.4 Other drug therapies

Along with the trials that we reviewed above, some other studies have also been designed in which PD-1, PD-L1 or the immune system are targeted. Atezolizumab, as an engineered humanized anti-IgG1, plus chemo-radiation is now under investigation in a trial which recruiting resectable esophageal cancerous patients (NCT03087864). Several trials are also underway evaluating the effect of ONO-4538 and TNO155 (in Combination with Spartalizumab or Ribociclib). TNO155 is used in non-small cell carcinomas, head and neck squamous cell carcinomas, esophageal SCCs, gastrointestinal stromal tumors, and colorectal cancers (NCT04000529). ONO-4538 is also being applied to unresectable advanced or recurrent gastric cancer (NCT02267343). Moreover, the potential effect of activated cytokine-induced killer cells armed with anti-CD3-MUC1 bispecific antibody is now under exploration on incoming advanced gastric cancerous patients (NCT03554395). M7824 targets pathway that prevents the immune system in patients with HPV induced cancers (the study patients are ongoing)(Figure 1).

4 PD-L1 and PD-1 as prognostic biomarkers

Despite improvements in therapy, the methods used for determining prognosis in gastrointestinal cancers remain suboptimal [GF4] [38]. There is a crucial need to form new therapy strategies [GF5]. In recent decades, a large amount of attention has focused on cancer immunotherapy.

Tumor development and its prognosis are related to host immune system and its response [39]. New findings in cancer immunology revealed the significance of the PD-1/PD-L1 interactions. A recent meta-analysis, focusing on PD-1/PD-L1 over expression as a prognostic biomarker graded by overall survival and disease-free survival scales, reported that PD-L1 over-expression adversely affect overall survival but not disease-free survival. Conversely, two studies included in their analysis show that PD-L1 over expression is a predictive factor of a favourable overall survival in esophageal cancer patients [40,41]. This controversy may be due to the fact that in special conditions, PD-L1 up-regulation might boost immune responses through unidentified receptors. It also leads to T-cell proliferation and secretion of specific cytokines, which in turn trigger strong anti-tumour responses [42]. Recently, the prognostic significance of the PD-1/PD-L1 pathway has been studied using other methods including immunostaining specimens obtained from patients. Immunohistochemistry (IHC) and antibody staining are two common methods for detecting PD-L1 over expression in a given tissue. Zhang *et al.* studied 344 specimens from ESCC patients using immunohistochemistry assessment of PD-L1 on tumour-infiltrating lymphocytes. Their results indicated that its expression was an independent factor for ESCC prognosis. Moreover, PD-L1 expression on lymphocytes had been detected in patients with improved survival [43]. In contrast, the result of the study of Mosome *et al.* revealed that high level of PD-L1 on the immune system was related with unfavourable prognosis in esophageal cancer [44]. Some studies have established a relationship between PD-L1 and other biomarkers, such as FOXP3+, MLH1, and HLA Class I [44–46]. Qing *et al.* showed that APE1 and PD-L1 expression were correlated. Most importantly, Qing *et al.* found that expression of APE1 and PD-L1 was considerably correlated to the intensity and extend of gastric tumour invasion

[47,48]. In addition, analysis indicated that PD-L1 and APE1 co-positivity was significantly associated with a poorer prognosis. Thus, APE1 upregulation in association with PD-L1 may be a prognostic factor of gastric cancer [47].

Chen *et al.* studied patients with tumour located in the upper part of the esophagus, highly transformed, without lymph node metastasis, or those at the early stage of disease have more chance of positive expression of PD-L1, which suggests that PD-L1 expression is a marker of less aggressive tumours [49]. In addition to PD-L1, some studies have reported that the presence of CD8⁺ T-cell infiltration in ESCC may be a positive prognostic factor with potential clinical implications [50,51]. According to previous studies, PD-L1 may boost immune evasion of tumour activating regulatory T cells and inactivating anti-tumour T-cells, which leads to poorer prognosis [52]. It has been reported that factors located in tumour microenvironment and anti-tumour treatment prompt PD-L1 regulation. Furthermore, experiments demonstrated that PD-L1 or PD-1 blockade could boost the tumour-specific T-cell responses and inhibit tumour cell proliferation, although intracellular signalling pathways still remain unclear [53]. Matta *et al.* showed that PD-L1 expression demands STAT3 activity, a transcription factor, which is associated with CD274 promoter, while the induction of PD-L1 by IFN- γ needs mitogen-activated protein-kinase (MAPK). Qing *et al.*, have concluded that MAPK and JAK/STAT pathways may be utilized for therapy of gastric carcinoma [47,53].

5 Mechanisms of Resistance to PD-1 and PD-1R

Interpreting resistance to anti-PD-1 and anti-PD-L1 is vital for the advancement of reversal procedures [54]. Resistance may be caused by gene mutations, PD-L1 over expression or

further processes that inhibit T-cell activation in tumour microenvironment [54,55]. Immune checkpoint therapies are ineffective in a considerable percentage of patients, and some early responders eventually show resistance to these treatments with disease recurrence [56]. Investigations show that an understanding the molecular mechanisms for the development of resistance can provide practical approaches which may improve clinical outcomes for patients [55]. It has been proposed that resistance can be reduced by accompanying therapies such as radiation therapy, immune stimulatory antibodies and vaccination that increase the antigenicity of the tumour [54]. Currently pembrolizumab and nivolumab as anti-PD1 antibodies and atezolimumab as an anti-PD-L1 antibody in addition to over 10 other anti PD-1/PD-L1 antibodies have been identified in different stages of clinical examinations in many diverse tumour(table1 shows more details) [55]. Resistance to anti PD-1/PD-L1 can be divided into 3 types including primary resistance (cancer does not react to immunotherapy), adaptive immune resistance (cancer is identified by the immune system but evades it by adjusting to its response), acquired resistance (cancer originally responds to immunotherapy but ultimately it recurs and advances). The cellular mechanisms and signalling pathways which lead to various types of resistance are different, and can be multi-factorial and overlapping among individuals [56]. Previous studies have shown that intracellular and extracellular factors are involved in the mechanisms of primary and adaptive resistance. The main tumour cell-intrinsic mechanism is expression or suppression of particular genes and pathways that can avoid infiltration or function of immune cell within the tumour microenvironment. This may occur at first presentation of PD-L1 to PD-1 which is considered as primary resistance or may develop later, which is called adaptive resistance. Major cell-intrinsic mechanisms are including lack of

antigenic proteins, antigen presentation, insensibility to T cells and genetic T cell exclusion. However, extrinsic processes in tumour cells that induce primary and adaptive resistance composed of other factors within the tumour microenvironment besides tumour cells, including absence of T cells, inhibitory immune checkpoints and immunosuppressive cells [GF6] [55]. The characteristic of cancer immunotherapy is the induction of long-term feedback, but there are tumours which respond for a while and then evade immunotherapy which is described as acquired resistance. For example Investigations have shown that almost a quarter to one-third of metastatic melanoma patients with significant responses to anti PD-1 therapy recur over time even though the therapy has continued [55,57]. Remarkably, mutations in tumours may also have relation with resistance to PD-1 blockade. For instance mutations in JAK1 or JAK2 (interferon-receptor) can result in the absence of response to IFN- γ -mediated anti-tumour effects [58,59]. Also mutation in β 2M leads to antigen presenting deficits [58–60]. Therefore, despite manifesting resistance to immunotherapy in different ways, identical or overlapping procedures let tumour cells to escape from anti-tumour immune responses [55].

5. PD-1/PD-L1 expression in GICs and tumor staging

Solid tumors usually express PD-L1 genes to avoid tumor-suppressing immune responses. Recent studies have reported histological data on PD-1/PD-L1 expression through different upper GIC microscopic subtypes. Starting with esophageal squamous cell carcinoma (ESCC), as Jiang et. al have reported three major microscopic patterns of PD-L1 expression has been found. These patterns include: (i) diffuse PD-L1 expression in the presence of TILs; (ii) regional expression of PD-L1 colocalized with TILs; and (iii) PD-L1 expression at the invasive front [61].

Additionally, Noh and colleagues managed to categorize ESCC pathohistological morphologies into four types abbreviated "TMIT". The items of this classification includes followings: 1) Type I: PD-L1+ with TILs, adaptive immune resistance; 2) Type II: PDL-1- with noTILs, immune ignorance; 3) Type III: PD-L1+ with no TILs, intrinsic induction; and 4) Type IV: PD-L1- with TILs, possible role of other immune tolerance mechanisms [62]. With respect to adenocarcinoma, PD-L2 expression was reportedly positive in nearly half of cases of Barrett's esophagitis that is a precursor of esophageal adenocarcinoma [63]. Of signet-ring cell adenocarcinoma, a study by Jin et. al found that approximately 40% of samples stained positive for PD-L1 . Moreover, PD-L1+ stained ESCC samples have been linked to significantly greater infiltration of immune cells of all types, predominated by CD8+ immune cell presence [64].

Considering the staging of ESCC, PD-L1 expression in tumor-infiltrating lymphocytes was found to be significantly associated with tumor N-stage. In addition, a significant association was established between positive rates of PD-L1 expression and metastatic gastric adenocarcinoma [65,66]. In line with these poor staging results, Chang et. al found that PD-L1 and PD-1 expression was significantly correlated with multiple adverse factors, including higher T-stage, diffuse type of Lauren histologic type, and lymphatic invasion [67]. To summarize, as of Derks et. al finding, expression of PD-L1 was significantly associated with higher tumor stages and showed poorer differentiation [63].

6. CONCLUSION

This review supports the concept that immune check point therapies like PD-1/PD-L1 inhibition can result in a significant improvement of anti-tumour activity of the immune system by means

of T-cell activation. Pembrolizumab and Nivolumab are some of the FDA approved inhibitors for tumour therapies for UGI malignancies that are still in progress. Pembrolizumab and Nivolumab are now under evaluation in two separate phase 3 trials. According to their beneficial effect in other cancers and also according to their notable implication on previous phase of trials, designing the next phases are recommended. Although SHR-1210 is not yet approved by the FDA for any other cancer, its significant efficacy in performed trials make promises to expect good results in ongoing phase 2 trials and make effort to complete the exploration in the next phases. A large and growing body of literature has been published on the use of combination therapies. Paying attention to what evidence shows, more investigations are needed to reach the best therapeutic plan. Despite the recent promising results on mentioned therapies, there are still some patients in whom these therapies do not work, and hence the need for personalized medicine (PM) in this field.

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Figure 1 anti PD1/PD1L drugs mechanism of action