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The therapeutic potential value of Cancer-testis antigens in immunotherapy of gastric cancer

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Abstract

Gastric cancer (GC) is the fourth most common cause of mortality and the fifth for incidence, globally. Diagnosis, early prognosis, and therapy remains challenging for this condition, and new tumor associated antigens are required for its detection and immunotherapy. Cancer-testis antigens (CTAs) are a subfamily of tumor-associated antigens (TAAs) that have been identified as a potential biomarker and target for immunotherapy. The CTAs-restricted expression pattern in tumor cells and their potential immunogenicity identify them as attractive target candidate in CTA-based diagnosis or prognosis or immunotherapy. To date, numerous studies have reported the dysregulation of CTAs in GC. Several clinical trials have been done to assess CTA-based immunotherapeutic potential in the treatment of patients with GC. NY-ESO-1, MAGE, and KK-LC-1 have been used in GC clinical trials. We review recent studies that have investigated the potential of the CTAs in GC regarding the expression, function, aggressive phenotype, prognosis, and immunological responses as well as their possible clinical significance as immunotherapeutic targets with a focus on challenges and future interventions.

Key words: Gastric cancer, Tumor-associated antigens, Immunotherapy, Cancer-testis antigens

Introduction

Gastrointestinal (GI) cancers (including gastric, colorectal, pancreatic, esophageal, and liver cancers) are among the most prevalent tumors diagnosed worldwide, and account for approximately 35% of all cancer-related deaths and a high rate of overall 5-year relapse [1, 2]. According to information from the American Cancer Society (2020), among the GI tract malignancies, gastric cancer (GC) is the fourth most common cause of cancer related mortality and the fifth for incidence globally with a higher rate among men than women in some countries, such as Iran [3]. There are various risk factors, carcinogens, genetic/epigenetic alterations, and epidemiologic patterns in GC formation and development [4, 5]. Among these factors in the Iranian population is *H. pylori* infection (the most prominent risk factor for GC), Epstein-Barr Virus (EBV), HTLV-1, alcohol, cigarette smoking, low economic level, food insecurity, family history, the prevalence of blood type A^+, diet, age (more common in people over 50 years), achalasia, unwashed hands after defecation, X-Ray dye exposure and CT imaging. The cellular and molecular processes involved in the development of GC, include: inflammation, cell adhesion, non-coding RNAs, self-renewal, cell cycle, DNA repair, apoptosis, signaling pathways, and transcriptional regulation [5, 6]. Lifestyle, epigenetic modifications through non-coding RNAs, abnormal immune responses, and inflammatory reactions can impact on the incidence of this disease [6]. The majority of GC patients are diagnosed at an advanced stage, often inappropriate for surgery; consequently, few patients have the chance for successful treatment due to early detection [7]. Despite advances in combination adjuvant and neoadjuvant chemoradiotherapy, however, a major number of patients still show a modest survival advantage, distant metastasis, and therapy resistance [2, 7]. Over the last few decades, there has been a considerable shift in the treatments of GC due to the advent of immune-targeted therapy [8]. The development of effective antitumor immunotherapies has been rapid to be used as a potential fifth pillar besides other conventional treatment of cancers [1]. Improvement in the understanding of the function and the molecular mechanisms of tumor antigens and the activity of immune cells have dramatically altered the field of immunotherapy [1]. The selected tumor-associated antigens (TAAs) for tumor vaccines can ameliorate antigen immunogenicity and therapeutic efficacy as well as prevent tissue-specific autoimmunity [9]. Tumor immunotherapy against tumor-related antigens has been identified as one of the most favorable treatment choices for GC patients, and depends on the T-cell responses to tumor antigen expression [10].

With the development of immunotherapeutic agents, cancer immunotherapy based on cancer/testis antigens (CTAs), as a subclass of TAAs, has demonstrated a significant area of investigation in clinical research. Subsequently, immune targeting of CTAs may have minor side effects due to the immuneprivileged property of testis for rare expression of HLA molecules [9, 11]. Moreover, CTAs are abnormally expressed in a wide variety of cancer tissues, such as breast, bladder, lung, head and neck, esophagus, and gastric cancers, and can serve as biomarkers for diagnosis/prognosis of cancer due to their specific expression patterns and strong *in vivo* immunogenicity [10, 12]. Accordingly, CTAs can be arbitrary targets for

immunotherapy, such as tumor vaccine, T-cell therapy (chimeric antigen receptor (CAR) T-cells and T-cell receptor engineering (TCR)), and immuno-check point inhibitors [13].

Hence, this review aimed to focus on the relationship between the expression CTAs in GC and its prognosis, targeting CTAs for diagnosis, and the function of CTAs in GC development. In subsequent sections, we outline immunotherapy in GC based on CTA-based immunotherapy and clinical trial studies.

Evidence acquisition

In order to obtain data on the expression of CTAs in GC, we performed a search of the PUBMED/MEDLINE databases with keywords: GC, cancer-testis antigen, immunotherapy.

Expression of CTAs in GC

Research has focused on the expression analysis of CTAs in GC tissue, serum, and cell lines using RT-PCR, western blotting, microarray, immunohistochemistry, methylation-specific PCR (MSP), and ELISA. Table 1 summarizes the relationship between CTAs expression, function, correlation of CTA expression with clinicopathological parameters as well as prognosis in GC samples. Based on Table 1, several CTAs are expressed and investigated in different types of GC samples.

Melanoma-associated antigen (MAGE) family

Human melanoma-associated antigen (MAGE) family classifies into five major subfamilies, including A, B, C, D, and E [14]. MAGE proteins are frequently expressed in germline cells and suppressed in somatic cells [15]. The dysregulation of mRNA and protein expression levels of MAGE family members modulate GC proliferation, invasion, and metastasis. Previous studies have revealed that the mRNA expression level of *MAGE-1/2/3* was upregulated in 38/41/31% of GC patients, respectively. The MAGE-A subfamily (including MAGE-A1, A2, and A3) was the most relevant CTAs that have been investigated by RT-PCR and immunohistochemistry (IHC) in GC tissue specimens and cell lines. *MAGE-A2* and *A3* were upregulated in GC cell lines, including SNU-16 and SNU-216 [16]. Furthermore, the protein and mRNA expression levels of MAGE-A3 were overexpressed in 62% and 50% of GC patients, respectively [17, 18]. The *H. pylori* infection is a well-established risk factor for GC development, which increases cell viability and proliferation via dysregulation of the β-catenin signaling pathway [19, 20]. It has been shown that the *MAGE-A3* expression is upregulated by *H. pylori* infection even after the elimination of *H. pylori* from the stomach. Therefore, *MAGE-A3* is considered for diagnosis and cancer immunotherapy, as it is persistently expressed in GC cells [21]. Due to the immunogenicity of CTAs, serum antibody against most immunodominant epitopes of MAGE-A3 has been detected in 66% of GC patients [22]. IgG antibody against MAGE-A3 protein was detected in 93% GC serum specimens with 44% sensitivity and 93% specificity. Positive rate antibody was related to stage III and IV of gastric tumor cells and lymph node metastasis. Additionally, it has been shown that *MAGE-A2*, *A4*, *C1*, and *C2* antigens were upregulated in

GC tissues; however, further functional studies are needed for these subfamilies [23, 24]. MAGE proteins have a crucial role in signaling pathways, which affect tumor cell progression, differentiation, and migration. The downregulation of *MAGE-A3* significantly reduced the cell proliferation and colony formation of cancer cells, while its ectopic expression enhanced cell growth, which result in poor clinical outcomes for GC patients [17]. Epigenetic modifications, including hypermethylation of promoter CpG islands, histone deacetylation, and DNA hypomethylation are critical factors in carcinogenesis and tumor progression [25-27]. It has been shown that the *MAGE-A3* hypomethylation was associated with poor prognosis and tumor progression in GC patients. The promoter demethylation of *MAGE-A3* (66%) and *MAGE-A1* (29%) were confirmed by MSP [28, 29]. The *MAGE-A1* expression was correlated with older ages, infiltration, vascular invasion, and nodal metastasis in differentiated advanced GC [30, 31]. It has been demonstrated that the promoter demethylation of *MAGE-A1* and *A3* was related to lymph node invasion, advanced stages, and poor prognosis. Therefore, it may be possible to use these subfamilies as a novel prognostic marker in GC. This novel insight into the pivotal role of CTAs in cancer development has provided new approaches to cancer therapy. Targeted therapies in combination with conventional cytotoxic treatments can significantly improve the survival rate of patients. Due to the role of MAGE-A1 and A3 in the progression and function of GC cells, targeting these proteins can be proper for designing novel drugs.

Chemotherapy is an essential part of GC treatment, consequently, the identification of predictive markers of response to chemotherapy is an urgent need. To the best of our knowledge, there are no reliable predictive markers to date for use prior to initiation of GC chemotherapy. Paclitaxel is the second-line of chemotherapy in advanced GC. Paclitaxel resistance was associated with increased expression of CTAs [32]. The MAGE-A1 protein expression resulted in a poor response to taxane-based chemotherapeutic agents including paclitaxel in GC patients. Moreover, the ectopic expression of *MAGE-A1* sensitized the TMK-1 GC cell line to paclitaxel [33]. Sitagliptin is an oral hypoglycemic drug that has a critical role in the inhibition of tumor cell progression [34, 35]. The overexpression of nuclear un-phosphorylated Yes-associated protein (YAP) is related to the stimulation of other oncogenes in GC. Sitagliptin inhibited the colony-forming ability and proliferation of GC cells [33]. It has been found that sitagliptin improves GC patients' prognosis through the inhibition of *MAGE-A3* following the suppression of *YAP* expression and activation of *AMPK*. Docetaxel is a cytotoxic chemotherapy agent that is used as a monotherapy or in combination with other drugs for GC patients. Pro-apoptotic genes, such as *P53*, *BAX,* and *P21* are involved in docetaxel-induced apoptosis [29]. The *MAGE-A3* downregulation reduced the expression level of pro-apoptotic proteins and tumor cell proliferation. Additionally, the *MAGE-A3* knockdown increased the susceptibility of GC cells to cell death signals. Thus, suppression of *MAGEA-3* increased sensitivity to docetaxel through *P53*, *BAX,* and *P21* [29]. Therefore, MAGE-A1 and A3 have the potential therapeutic to be a novel target for GC treatment or a predictive marker for response to chemotherapeutic agents [17].

Cancer-associated gene (CAGE)

The CTA cancer-associated gene (*CAGE*) was initially identified in the sera of GC patients [36]. The *CAGE* promoter hypomethylation has been identified in 80% of GC patients, which can be associated with its expression. Since the *CAGE* hypomethylation was related to GC development and progression; it would introduce as a diagnostic marker in GC [37]. The expression of CAGE mRNA and protein were detected in 73% of GC tissue and sera samples, respectively [36, 38]. Moreover, *CAGE* is expressed in exosomes derived from the AGS cell line and could act as a mediator of anticancer drug resistance that is closely associated with antiapoptotic effects and autophagic flux [39]. It has been shown that the *CAGE* expression is regulated by autophagic efflux via a negative feedback loop with sponging miR-302-5p [39]. Furthermore, the CAGE-miR-181b-5p-S1PR1 axis was negatively correlated with anticancer drug resistance and autophagic flux; consequently, this axis can be used as a target for anticancer drug development [39].

Developmental pluripotency associated-2 (DPPA2)

The CTA developmental pluripotency associated-2 (*DPPA2*) or CT100 is a member of cancer-embryo antigens or developmentally restricted differentiation antigens (DRDAGs) that express in the human germ line, pluripotent embryonic cells, and a significant subset of tumor cells [40]. *DPPA2* was upregulated in 42% of GC tissue compared to adjacent normal tissue samples as its overexpression was associated with lymph node metastasis and tumor aggressiveness, supporting the role of *DPPA2* in GC progression and introducing it as a marker for GC invasion and metastasis [41].

G melanoma antigen (GAGE) family

The GAGE family, a subset of CTAs with 16 genes (including GAGE1/2A/2B/2C/2D/2E/10/12C/12D/12E/12F/12G/12H/12I/12J/13), is specifically expressed in germ cells and modulate apoptotic regulators; so their dysregulation is implicated in a wide range of malignancies [40, 42]. The dysregulation of GAGEs is confirmed in neuroblastoma and esophageal cancer and is related to poor prognosis [43, 44]. The overexpression of GAGEs was found in mRNA and protein levels in the intestinal-type of GC tissue samples [45]. The *GAGE12* overexpression increased growth, migration, tumor sphere formation, invasion, and metastasis in GC cell lines (such as SNU-1, 16, and 638), indicating the critical role of *GAGE12* in modulating the expression of genes involved in GC metastasis and development [46].

Kita-Kyushu lung cancer antigen-1 (KK-LC-1)

The CTA Kita-Kyushu lung cancer antigen-1 (*KK-LC-1*) or CT83 is expressed in various types of germlines and tumor tissues but not in normal tissues [47, 48]. It has been reported that the *KK-LC-1* expression is found in 80% of GC tissue specimens [18, 49, 50]. Evaluation of the KK-LC-1 protein level with monoclonal antibody was detected in 81% of GC tissue samples, proposing KK-LC-1 valuable diagnostic marker [51]. Helicobacter pylori (*H. pylori*) infection following atrophic gastritis is the risk factor for the

development of GC. The *H. pylori* infection upregulated the *KK-LC-1* expression during the initiation and development of GC [18]. There is a classification for GC patients based on the detection of anti-*H. Pylori* IgG and pepsinogen (PG) I/II in serum (A, B, C, and D groups). The *KK-LC-1* overexpression was associated with group C patients who had atrophic PG and positive *H. pylori* IgG [52]. It has been indicated that *KK-LC-1* is expressed in cancerous and precancerous lesions, including the pyloric gland area where *H. pylori* preferentially colonize. Interestingly, the *KK-LC-1* expression was detected in 94% of anti-*H. Pylori* IgG positive patients. Moreover, the mRNA expression level of *KK-LC-1* was detected in 80% of stage I GC tissues, 67% and 32% in patients with and without intestinal metaplasia, respectively [11, 50]. Taken together, these data suggest the role of KK-LC-1 at the early onset of tumor formation, premalignant lesions, tumor progression, and development, which may provide KK-LC-1 as a potential candidate for early diagnosis and immunotherapeutic target in GC [11, 18, 51].

LEM Domain Containing 1 (LEMD1)

The CTA *LEMD1* is highly expressed in colorectal and prostate cancers, so that *LEMD1* may be a diagnostic biomarker [53, 54]. Moreover, the protein expression level of LEMD1 was increased in GC compared with non-cancerous tissue samples. The upregulation of LEMD1 was indicated in GC cell lines of BGC823, SGC7901, MKN45, and MGC803 through western blot analysis, while the mRNA expression level of *LEMD1* was shown in 51% of GC tissue in comparison with adjacent normal tissue samples [55]. The *LEMD1* overexpression was correlated with increased tumor size and decreased survival rate in GC patients [55]. The overexpression of *LEMD1* promoted the phosphorylation of PI3K and AKT proteins to activate the PI3K/AKT signaling pathway in GC cells, leading to cell growth and proliferation via modulating the cell cycle and apoptosis. Thus LEMD1 regulated tumor cell growth via PI3K/AKT targeting [55].

Maelstrom (MAEL)

The CTA maelstrom (*MAEL*) gene plays an essential role in spermatogenesis through repressing transposons [56]. The MAEL functions as a double-edged sword (either tumor suppressive or oncogenic role) in various malignancies, including glioblastoma, gastric, invasive breast, and lung cancers; despite its relatively limited expression in testis [56-58]. However, MAEL acts as a CTA and upregulates in tumor tissues [59]. DNA methylation regulates the *MAEL* expression in breast and colorectal cancers, subsequently, the promotor hypomethylation upregulates most CTAs [57, 59, 60]. The *MAEL* expression was shown a substantial inverse correlation with DNA methylation in GC, implying the overexpression of *MAEL* results from DNA hypomethylation in GC [61]. The increased mRNA expression of *MAEL* was significantly associated with the early stages of tumor development, *H. pylori* infection, low grade of tumor cells, and poor survival, suggesting *MAEL* can serve as a biomarker for GC. Interestingly, the overexpression of *MAEL* was related to tumor invasion towards the marginal lymph nodes [62]. Moreover, the *MAEL* upregulation led to self-renewal and suppress tumor differentiation in the primary stages of tumor

cells, proposing *MAEL* as a cancer stem cell marker. Silencing of *MAEL* inhibited cell proliferation, migration, and tumor growth *in vivo* and *in vitro*, indicating the role of *MAEL* as an oncogene [62]. ILKAP is a subunit of the MAEL protein complex and acts as a tumor suppressor via dephosphorylation of its substrates, such as p38 MAPK, CHK1, and RSK2 [61]. Interaction of MAEL with ILKAP has improved lysosome-dependent degradation of ILKAP. The *ILKAP* overexpression suppressed the oncogenic role of MAEL *in vivo* and *in vitro* [61].

Mitotic centromere-associated kinesin (MCAK)

Mitotic centromere-associated kinesin (*MCAK*), a member of kinesin-13, is expressed throughout the cell, and is found in the centromeres, kinetochores, and spindle poles [63]. Members of kinesin family (KIF) are microtubule-dependent molecules, which contain the motor catalytic and coiled-coil domains and involve in intracellular transport and cell division [64-66]. *MCAK* is expressed at a high level in meiotic and proliferating cells of the testis and ovary. According to, MCAK may be a CTA and used as a target in immunotherapy [67]. The overexpression of *MCAK* was indicated in 66% (43 out of 65) of GC tissues compared with adjacent normal tissues. Moreover, there was an association between the *MCAK* overexpression with tumor invasion, lymph node metastasis, poor prognosis, cell proliferation, and tumor growth, suggesting the oncogenic role of *MCKA* in GC [68].

New York esophageal squamous cell carcinoma-1 (NY-ESO-1)

The CTA New York-esophageal-1 (NY-ESO-1) was discovered in esophageal carcinoma for the first time based on its capacity to induce a detectable antibody response in cancer patients [69-71]. The *NY-ESO-1* expression on tumor cells and following immunological response, making it a prospective target for cancer vaccines [72, 73]. The mRNA expression level of *NY-ESO-1* was approximately between 17-24% of GC patients [11, 18, 49]. The NY-ESO-1 protein level was observed in 8% of GC patients using immunohistochemistry analysis [24]. Moreover, it has been revealed that 50% (6 out of 12) of GC patients indicated antibody against NY-ESO-1 in the serum samples [74]. Furthermore, the NY-ESO-1 antibody was detected in 11% (41 out of 363) of GC patients with advanced stages of tumor development by ELISA analysis [75]. The NY-ESO-1 humoral immune response combined with CEA and CA-19 was a valuable marker for the detection of stages III and IV of GC. Additionally, its antibody level was decreased in GC patients without recurrence after surgery and the patients who received only chemotherapy continued to indicate the NY-ESO-1 expression in serum samples [75]. A large-scale serological study reported that the NY-ESO-1 antibody was detected in 10% of GC serum specimens. Despite the immunogenicity and the NY-ESO-1 expression level in several tumor tissues, this antigen cannot be utilized as a marker or therapeutic target in GC. Further studies and more robust evidence are needed to investigate this antigen in GC.

PDZ binding-kinase (PBK)

The CTA PDZ binding-kinase (*PBK*) or TOPK (T-lymphokine-activated killer cell-originated protein kinase) is a serine/threonine protein kinase that is significantly expressed in breast, gastric, and lung cancers [76-78]. PDZ is formed by combining the first letters of three proteins, including domain-post synaptic density protein (PSD95), Drosophila disc large tumor suppressor (Dlg1), and zonula occludens-1 protein (zo-1). PDZ domains have an important role in anchoring receptors to cytoskeletal components [79]. The overexpression of *PBK* was found in testis and GC cell lines, including Kato III, NUGC4, HGC27, and MKN45. The *PBK* upregulation was indicated in 17% (24 out of 144) primary GC tissue specimens, indicating PBK is a CTA and acts as an oncogene for GC development [80]. The *PBK* expression was identified in the cytoplasm and nuclei of gastric adenocarcinoma cells by immunohistochemical analysis [81]. The nuclear expression of *PBK* was correlated with poor prognosis and advanced stages of GC development, while its cytoplasmic expression was correlated with early stages of GC development [81]. The protein expression of PBK was significantly associated with TNM staging and recurrence rate in GC patients. Moreover, the PBK/TOPK overexpression was related to venous invasion, tumor depth, and recurrence rate of GC patients [80]. Although, the *PBK* expression promoted migration and invasion of GC cell lines, including SNU638 and AGS, however, its expression was not sufficient for cell proliferation [81]. PBK reduces the expression of *P53* and *P38-MAPK*, which in turn, suppresses tumor cell mortality [82-84]. The interaction of PBK with the PI3K/PTEN/AKT axis promoted cell migration, introducing the oncogenic role of PBK via P53 and the PI3K/AKT pathways in GC [80].

Placenta specific-1 (PLAC1)

Human placenta-specific peptide (PLAC1) is expressed in human tumors and germ cells [69]. The PLAC1 protein expression level was found in 61% of GC tissues using immunohistochemical analysis [85]. Moreover, the *PLAC1* had a high expression level in GC tissue specimens, SGC-7901, and MGC803 cell lines [86]. The *PLAC1* expression was associated with poor prognosis and decreased survival rate [85]. The overexpression of *PLAC1* was found in some specific types of GC patients, including patients with intestinal types and non-infected with *H. pylori*. Accordingly, PLAC1 can apply as a potential marker for the diagnosis, prognosis, and treatment of GC [86]. The *PLAC1* overexpression had a critical role in cell proliferation via the AKT/GSK3/cyclin D1 signaling pathway and it was shown that AKTi could promote function effects in GC cell lines [86].

SCRN1 (Secernin 1)

The CTA *SCRN1*, as a cytosolic protein, involves exocytosis in mast cells [71]. The increased mRNA expression of *SCRN1* is revealed in the testis, ovary, and gastric (9 of 11 GC tissue samples) with much less abundant in normal adult tissues, proposing the function of *SCRN1* as a CTA. It has been provided that the increased expression of *SCRN1* is associated with increased tumor cell proliferation, tumor growth, and colony formation [87].

V-set and immunoglobulin domain containing 1 (VSIG1)

The CTA V-set and immunoglobulin domain containing 1(VSIG1) is a newly identified member of the junctional adhesion molecules (JAM) family [88]. The VSIG1 mRNA and protein levels were found in testis, normal gastric tissue, and some types of tumor tissues, such as gastric, esophageal, and ovarian [89]. The mRNA and protein expression levels of VSIG1 were downregulated in GC tissues and upregulated in the membranes of non-cancerous gastric glandular epithelial cells in the cardia, corpus, and antrum as well as the cytoplasm of those cells [89-91]. The *VSIG1* expression was detected in some GC cell lines, including SGC7901, MGC803, HGC27, MKN45, and AGS. In more than half of GC samples were indicated negative VISG1 expression through immunohistochemistry and western blotting [91]. *VSIG1* inhibits proliferation, migration, and invasion of cancer cells. Thus, VSIG1-expressing GC patients had a better prognosis and higher overall survival in comparison with VISG1-downregulated patients [90, 91]. The downregulation of *VSIG1* was related to tumor size and infiltration but not to nodal metastasis, suggesting that *VSIG1* may accelerate invasion and metastasis of GC [91]. According to the reduced expression of *VSIG1* in GC and its association with more malignant tumor phenotypes and poor prognosis, *VSIG1* may have a tumor suppressor role in GC cells [90]. Taken together, the results of previous studies suggest *VSIG1* as a gastric-specific marker.

Function of CTAs in GC development

CTAs may have several roles in the pathogenesis and development of GC. These CTAs have demonstrated their effects on some carcinogenesis levels, the known function in the CTAs examined in GC can be classified into the following steps, including invasion, metastasis, cell proliferation, anti-apoptotic effects, and stem cell maintenance. Moreover, CTAs affect signaling pathways involved in GC that are included β-catenin, AKT/GSK3/cyclin D1, PI3K/AKT, cell cycle, and apoptosis signaling pathways [20, 55, 86].

Invasion and metastasis

CTAs have been revealed their role in different steps of invasion and metastasis (such as loss or lack of cell-cell adhesion, interruption of the basement membrane, invasion, intra and extravasation) in various malignancies [92]. Various CTAs that are expressed in GC, such as MAGE-A1, DPPA2, GAGE12, MEAL, MCAK, PBK, and VSIG1, have been revealed to increase the potential of cancer cells invasion and EMT [30, 31, 41, 46, 62, 68, 80, 91]. In addition, MAGE-A1, MAGE-A3, DPPA2, GAGE12, MEAL, MCAK, and VSIG1 are among CTAs that have a role in metastasis of tumor cells [31, 41, 46, 62, 68, 80, 90].

Cell proliferative and anti-apoptotic effects

MAGE-A3 has been shown to promote cell proliferation, cell growth, and colony formation of tumor cells through Yes-associated protein (YAP)/AMPK pathway inactivation [17]. Another study has shown that CAGE role in decreasing antiapoptotic effects has been proposed to be exerted through the miR-181b-5p-

S1PR1 axis [39]. Moreover, GAGE12, MCAK, MEAL, and SCRN1 have been shown tumor cell growth, proliferation, and colony formation in GC cells [46, 62, 68, 87]. LEMD1 as a CTA is expressed in GC cells via activating the PI3K/AKT signaling pathway and modulating the cell cycle and apoptosis [55].

Stem cell maintenance

It has been identified that a tiny part of tumor cells has cancer stem cells (CSCs) features, such as self-renewal capacity, maintenance of tumor growth, differentiation, promotion metastasis, tumor cell heterogeneity, therapeutic resistance, and relapse [93, 94]. The presence of CSCs has been documented in GC [94]. A study has revealed the expression of MEAL has a self-renewal capacity in GC samples and inhibits tumor differentiation in the primary stages of tumor growth, consequently it can be introduced as a CSCs [62].

CTA expression and GC patient's prognosis and diagnosis

The expression of some CTAs has been related to the clinical outcomes of patients. The expression of *MAGE-A1* and *A3* has been shown to correlate with poor clinical outcomes, poor prognosis, and tumor progression of GC patients, consequently, MAGE-A subfamily suggests as markers for advanced GC [30]. The expression of *CAGE*, *DPPA2*, *MCAK*, *NY-ESO-1*, *PBK*, *PLAC1*, *LEMD1*, and *VSIG1* have been demonstrated in GC metastasis and not in primary tumors that are associated with poor prognosis and would introduce as a diagnostic marker in GC [41, 55, 68, 75, 80, 85, 90]. Moreover, *KK-LC-1* and *MAEL* as potential candidates have correlated to poor survival rates and can provide for early diagnosis in GC [51, 62].

Immunogenicity of CTAs in GC

Serum antibodies directed against the CTAs potentiates its significance as the key biomarker and immunotherapeutic target, which indicates the antitumor immune reaction levels associated with the clinical response [95]. Numerous CTAs (such as NY-ESO-1, MAGE-A4, and XAGE) is modulated by epigenetic changes and revealed on the X chromosome. Consequently, inactivation of X chromosome leads to aberrant epigenetic alterations and dysregulation of CTAs, which results in tumor cell evasion from the immune system [96]. The spontaneous humoral and/ or cellular immune responses against some CTAs have been restricted in GC patients and not indicated in healthy subjects. In a serum screening assessment of 210 GC patients and 116 healthy controls, serum-specific IgG antibody responses were detected in 65.71% of GC patients and none of the control group. The specificity of MAGE-A3 IgG detection for serological diagnosis of GC was 97.67%, while the sensitivity was 65.71%. Moreover, a high level of specific IgG antibody to the MAGE-A3 protein was produced in immunized mice, proposing its application as a potential target for serological diagnosis and design of cancer vaccines [22]. In another study, the sera survey of 5 GC patients identified the CAGE expression associated with its promoter methylation [36, 37].

Moreover, the expression of CAGE was shown in 12% early-stage of sera GC patients, suggesting the role of CAGE as a target of immunotherapy [97]. In relatively recent studies, the monoclonal antibody (Kmab34B3) was detected the protein expression level of KK-LC-1 in GC cell and pyloric gland specimens [51]. Moreover, in serum screening investigation of GC patients, anti-H. pylori IgG responses were shown in 94% of KK-LC-1-positive GC patients compared with KK-LC-1-negative patients, proposing KK-LC-1 as a diagnostic marker and immunotherapeutic target [18]. Following serological analysis of recombinant cDNA expression libraries (SEREX) led to the NY-ESO-1 gene identification and its protein expression level was detected in testis and tumor samples by immunoreactivity of anti-NY-ESO-1 monoclonal antibody ES121. The level of IgG antibody humoral immune response to the NY-ESO-1 protein was detected in sera from 11.1% of GC patients, proposing NY-ESO-1 as a valuable marker for advanced GC [75].

CTAs in cancer immunotherapy

New approaches for treatment of GC such as cancer immunotherapy (including cancer vaccines, adoptive T cell transfer, monoclonal antibodies, checkpoint inhibitors/immune modulators, cytokines, kinases, and mechanistic target of rapamycin (mTOR) inhibitors) particularly those with distant metastasis are urgently needed [98]. Immunotherapy may be applied to many types of cancers using tumor antigens (tumor-specific or associated antigens) as targets [9]. With the recent progress in the field of biomarkers (tumor antigens) and clinical experience, cancer immunotherapy can have a critical role in the survival of many GC patients [99]. The detection of the limited expression patterns of CTAs, the specific humoral and cellular responses along with the inducible immune responses against certain CTAs have provided the evidence for the suitability of CTAs as ideal therapeutic targets for immunotherapy [100, 101]. Subsequently, some CTAs that have tumor-restricted expression and induce immune system responses could enter clinical trials studies for improving the survival of patients [102, 103].

Clinical trials of CTA-based immunotherapy in GC

The restricted expression of CTAs in tumor cells and some germline cells make them suitable candidates for cancer vaccine and T cell therapy to induce specific cellular and humoral immune responses [9]. Cancer vaccine efficiency is associated with the immunogenicity of chosen antigen and nonspecific immunostimulatory adjuvant that promote the immune responses [9, 104]. According to the type of antigen, cancer vaccines are classified into vaccines targeting a single antigen or multiple antigens or in combination with chemotherapy to enhance specificity and clinical efficacy [9]. T cell therapy refers to the extraction of T cells from cancer patients, expansion of them, and reinfusion of them into patients alone or in combination with a chimeric antigen receptor (CAR) or a specific T cell receptor (TCR) [105]. Table 2 summarizes the clinical trials conducted on GC patients. MAGE-A4, NY-ESO-1, and KK-LC-1 are CTAs that utilize for cancer vaccine immunotherapy in GC. In two phase I clinical trials in GC patients, the MAGE-A4c1032T

and ADP-A2M4CD8 cells alone or in combination with low dose radiation and Nivolumab, respectively, were utilized in HLA-A2+ participants to assess safety, tolerability, and antitumor activity. In a Phase I trial of T cell therapy for KK-LC-1 positive epithelial cancers (such as gastric, breast, cervical, and lung), KK-LC-1 TCR T cells plus IL-2 (Aldesleukin)/ Cyclophosphamide/ Fludarabine were administered to patients. The other clinical trials were operated based on tumor-associated antigen (TAA) of NY-ESO-1. For example, two clinical trials were conducted by autologous T cells transduced with affinity-enhanced NY-ESO-1 TCR (TAEST16001) in combination with chemotherapy and EGFRvIII/DR5/NY-ESO-1/Mesothelin CAR T/TCR-T cells in positive HLA-A2*02:01 subjects for evaluating the safety, tolerability, and clinical response to T-cell infusion. Moreover, in a phase I clinal trial, the HER2 and NY-ESO-1 vaccine was combined with OK-432 (Picibanil) as an immunoadjuvant for evaluating immune responses including HER2 and NY-ESO-1 specific IgG and T cells. Finally, a non-randomized and phase I clinical trial of mTOR inhibition with rapamycin for enhancing intranodal dendritic cell vaccine was performed in patients with NY-ESO-1 expressing solid tumors to assess the safety and toxicity. However, the results from these trials have not been unpublished.

Limitations of CTAs

Immunotherapy based on CTAs is a potential intervention for inducing antigen-specific immune responses in GC patients due to their dysregulation in a range of malignancies and normal testis tissue [106]. Consequently, targeting these antigens for immunotherapy and cancer systemic diagnosis has few side effects and may be advantageous due to their specific pattern of expression, respectively [11]. However, there are relatively few clinical trials and may reflect some limitations of CTAs in GC-based immunotherapy and prognosis. The expression of CTAs is rarely indicated in lymphoma, gastric, colon, and renal cancers as well as tumor cells that express CTAs desire to co-expression of several CTAs, indicating a coordinated CTA-expression plan instead of the independent expression of them [69]. However, some epigenetic alterations such as promoter demethylation are able to enhance the expression of CTAs for ameliorating tumor diagnosis by the immune system [107-109]. Among of obstacles to CTAs-based immunotherapy can be mentioned to the tumor microenvironment that promotes or prevent the immune system when CTAs presentation [110]. Identification of the role of regulatory T cells in eliciting T cells immune responses upon CTAs excitation, the association of HLA to CTAs responses, and recognition of epitope immunodominance/eclipse/tolerance could help appropriate immune-based therapies targeting CTAs in GC [111].

Conclusion

CTA expression is restricted to the testis and tumor tissues, including GC. However, their expression is low in GC, but the immunogenic potential remains good. The most CTAs functions are still not understood in GC tumorigenesis, however, advances in the identification and evaluation of new CTAs and their

immunogenicity can help to improve impressive anticancer immunotherapies. Although, it has been indicated that CTAs apply oncogenic effects in GC via enhancing cell proliferation, growth, migration, invasion, metastasis, anticancer drug resistance, and autophagy, and therefore CTAs have been proposed as appropriate immunotherapeutic targets. The spontaneous cellular or humoral immune responses against CTAs as a group of TAAs that are expressed in tumor tissues are more frequent compared with over other TAAs such as differentiation antigens. CTAs are identified as one ideal targets for tumor vaccine and T cell therapy, and several clinical trials have been started in GC. Phase I clinical trials targeting the MAGE-A4, NY-ESO-1, and KK-LC-1 have been initiated in GC. However, future studies are required in the reorganization of novel CTAs, their functions, their biological roles, the fundamental mechanisms of CTAs, the best formulation for vaccines and adjuvants, RNA or peptide sequence of vaccines as well as appropriate inclusion and exclusion criteria for choice of patients to access the best target for immunotherapeutic approaches. Nevertheless, the role of most CTAs in GC development is not unknown and needs comprehensive studies in the future.

Future perspective

The key step in the successful use of immunotherapy is discovery of suitable target antigens. Considering the CTAs expressed in GC cells have not yet been fully discovered and their function is not known as well as they may not be well identified by the immune system; consequently, further challenges on the road extract the most appropriate CTA-based immunotherapy approaches exists to induce an immune response. Accordingly, CTA-based vaccines still need to be improved to provide more effective. Considering the heterogenicity in GC, the heterogeneous expression of CTAs in cancer specimens, and the different frequencies of their expressions in patients, a combination of markers or multi-antigenic panel improve prognosis and immunotherapy (the immune response against CTAs) rather than a single marker. Novel strategies based on cell-mediated immunotherapy are recently developed to induce immune responses against tumor antigens. Moreover, high-throughput technologies are facilitated the patient samples screening for identifying the immunogenic, specific, and sensitive prognostic and diagnostic tumor antigens biomarkers; consequently, prevail immune evasion. Design an optimal personalized therapy strategy based on the antigen-specific humoral and cellular responses against CTAs would develop immunotherapeutic approaches for the patients.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

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Competing interests

The authors declare that they have no competing interests.

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Table 1: The expression of Cancer-testis antigens and their relationship with clinicopathologic parameters and their function.

CTA of interest	mRNA exp. in tissue: Case (Total)	Protein exp. in tissue: Case (Total)	Exp. in cell line	Antibody In serum: Case (Total)	Method of CTA assessment	CTA exp., clinicopathological parameters, and prognosis	Function of CTA	Functional study	Ref.
CAGE	MSP: 50 (64) RT-PCR: 9 (16), 17 (19)	-	AGS	5 (5)	Immunoblot Immunoprecipitation Methylation-specific polymerase chain reaction (MSP) RT-PCR	CAGE and miR-302b-5p is regulated by each other	Increase anticancer drug resistance, autophagy, and invasion	CAGE-miR-181b-5p-S1PR1 axis is a key regulator of chemoresistance and autophagy. CAGE binds to beclin1 and induces autophagy	,37 ,36] [39
PLAC1	-	73 (119)	SGC7901 MGC803	-	Western blot Immunohistochemistry	PLAC1 overexpression is opposed to survival rate, intestinal type GC, and presence in *H.pylori* non-infected patients.	Increase cell proliferation	Increased tumor cells proliferation via the AKT/GS-3β/cyclin D1 signaling pathway	[85, 86]
MAGE-A3	34 (82) 12 (25)	41 (66)	AGS HGC-27 MKN45	-	Immunohistochemistry RT-PCR Western blot	MAGE-A3 expression is related with tumor cells differentiation, lymph node metastasis, and poor prognosis.	Increase cell proliferation, differentiation, and colony formation	Sitagliptin inhibits MAGE-A3 through YAP suppression and AMPK activation	[11, 17, 18]
MAGE-A3	-	-	SNU620 AGS AZ521 SNU638 NUGC4 NUGC3 OCUM1 MKN1 KATOII	-	RT-PCR Western blot Immunohistochemistry Methylation-specific polymerase chain reaction (MSP)	Hypo-methylation of MAGE-A3 is associated to poor prognosis.	-	Suppression of MAGE-A3 is increased sensitivity to stress and docetaxel through p21, Bax, and p53.	[29]
MAGE-A3 (three epitope)	-	-	-	E1:138 (210) E2: 100 (210) E3:75 (210)	ELISA Western blot PCR	-	-	-	[22]

MAGE-A3	-	-	Meth-A cells	-	RT-PCR	-	*H.pylori* infection is induced MAGE-A3 expression	-	[21]
MAGE-3	22 (51)	-	SNU-1 SNU-16 SNU-484 SNU-601 SNU-216	-	RT-PCR Immunohistochemistry	-	-	-	[16]
MAGE-A1	22 (82) 11 (25) 17 (49)	-	-	-	RT-PCR	-	-	-	[11, 18, 49]
MAGE-A1	-	4 (41)	TMK-1 GC	-	Immunohistochemistry Western blot Methylation-specific polymerase chain reaction (MSP)	MAGEA-1 expression is a predictive marker for response to taxane-based therapies.	Expression of MAGE-A1 is sensitized the TMK-1 GC cell line to paclitaxel.	-	[33]
MAGE-A4	17 (82) 5 (25)	-	-	-	RT-PCR Immunohistochemistry	-	-	-	[11, 18, 23, 24, 112]
MAGE-1	24 (101) 16 (51)	44 (135)	SNU-16 SNU-484	-	RT-PCR Immunohistochemistry	Overexpression of MAGE-1 is associated with older ages, infiltration, vascular invasion, and nodal metastasis in differentiated advanced gastric cancer.	-	-	[16, 31, 74]
MAGE-A1 **MAGA-A3**	22 (84)	-	MKN1 MKN7 MKN28 MKN74 MKN45 KWS-I KATO-III TSG11 ECC10 ECC12	-	Methylation-specific polymerase chain reaction (MSP) RT-PCR	Demethylation of MAGE-A1/3 promoters is related to lymph node invasion, advanced stages, and poor prognosis.	-	-	[28]
MAGE-C2 (CT10)	14 (101)	-	-	1 (50)	RT-PCR	-	-	-	[24, 74]
MAGE-C1 (CT7)	6 (101)	-	-	3 (50)	RT-PCR	-	-	-	[24, 74]

KK-LC-1	63 (77) 6 (11) 66 (82) 66 (83)	-	-	-	RT-PCR Immunohistochemistry	*H.pylori* infection, was correlated with group C of ABCD classification and intestinal metaplasia. Kmab34B3 detected KK-LC-1 protein within tumor cells.	KK-LC-1 is overexpressed in early and advance stage of gastric cancer.	-	[11, 50, 51, 113]
KK-L-1	40 (49)	-	-	-	RT-PCR	-	-	-	[49]
MAEL	3 (4)	3 (4)	HGC-27 AGS KATOIII	-	RT-PCR Immunohistochemistry	MAEL mRNA expression was related to poor prognosis.	Promoted cell proliferation, colony formation, migration, invasion, and the growth *in vivo*.	Increased degradation of ILKAP and phosphorylated p38, CHK1, and RSK2 subsequently. ILKAP overexpression *is* suppressed the MAEL oncogenic roles *in vivo* and *in vitro*.	[61]
LEMD1	25 (49)	-	BGC823 SGC7901 MKN45 MGC803	-	RT-PCR Western blot	LEMD1 expression is correlated to tumor size and poor survival. It also significantly upregulated in tumor tissues.	LEMD1 expression is promoted tumor cells growth.	LEMD1 promotes cell growth through PI3K/AKT signaling pathway	[55]
SSX1	23 (101)	-	-	-	RT-PCR	-	-	-	[74]
SSX2	3 (101)	-	-	-	RT-PCR	-	-	-	[74]
SSX4	18 (82) 4 (25) 27 (101) 21 (102)	-	-	-	RT-PCR	-	-	-	[11, 74, 114]
NY-ESO-1	7 (49)	-	-	-	RT-PCR, Immunohistochemistry	-	-	-	[18, 24, 49, 112]
NY-ESO-1	6 (60) 12 (101)	19 (60)		41 (363) 6 (12)	RT-PCR Immunohistochemistry ELISA	Positive antibody is correlated to outcome after surgery.	Presence of antibody increases with progression of cancer.	-	[74, 75]
PBK/TOPK	-	24 (144)	Kato III NUGC4 HGC27 MKN45	-	RT-PCR Western blot Immunohistochemistry	Overexpression of PBK/TOPK is related to poor prognosis of GC patients.	PBK/TOPK overexpression is related to venous invasion, tumor depth, and recurrence rate.	PBK/TOPK is an oncogene acting through p53 and PI3K/AKT pathways.	[80]

PBK	48	385	SNU638 AGS	-	RT-PCR Immunohistochemistry	PBK expression is associated with the depth of invasion, lymph node metastasis, and lower survival rates in GC.	PBK overexpression is suppressed cell migration and invasion.	-	[81]
VSIG1	23 (30)	L219 (362) L18 (26) WB, 126 (232) IHC	MKN45 SGC7901 MGC803 HGC27 AGS	-	RT-PCR Immunohistochemistry Western blot	Suppression of VSIG1 is related to poor prognosis. Low expression of VSIG1 is related to tumor size and infiltration (T&M) but not with nodal metastasis (N). VSIG1 negative cases has worse OS.	VSIG1 expression is suppressed proliferation, invasion, and migration of cancer cells.	VSIG1 is a tumor suppressor gene.	[90, 91]
DPPA2	-	23 (55)	-	-	Immunohistochemistry	Expression of DPPA2 was associated with aggressiveness of tumor	-	-	[41]
GAGE12	-	-	SNU-1 SNU-16 SNU-638	-	RT-qPCR	-	GAGE -12 expression is promoted tumor cells development and metastasis.	-	[46]
GAGE	3 (24) 15 (60)	-	-	-	Immunohistochemistry RT-PCR Southern blotting	Expression of GAGE is correlated to intestinal type of GC, tumor invasion, and poor prognosis.	-	-	[24, 45, 114]
CT45	-	4 (50)	-	-	Immunohistochemistry	-	-	-	[24]
NXF2	-	3 (50)	-	-	Immunohistochemistry	-	-	-	[24]
SAGE-1	-	4 (50)	-	-	Immunohistochemistry	-	-	-	[24]
TEKT5	4 (10)	-	-	-	RT-PCR	-	-	-	[115]
BCP-20 (FBXO39)	-	-	-	1 (24)	ELISA	-	-	-	[116]
CCDC62-2	-	-	-	6 (104)	ELISA	-	-	-	[117]
STK31	-	-	AZ521 NUGC3 KATOIII MKN1 MKN28	-	-	-	-	-	[118]

			MKN94						
OIP5	34 (58)	-	-	-	RT-PCR	-	-	-	[68]
MCAK	43 (65)	-	AZ521 KATO3 MKN1 MKN7 MKN28 MKN45 MKN74 NUGC3 NUGC4 SH10TC	-	RT-PCR Immunohistochemistry	Expression of MCAK is associated with lymphatic invasion, lymph node metastasis, and poor prognosis.	Promoted tumor cells proliferation and invasion.	-	[68]
SCRN1	9 (11)	-	MKN1 MKN28 MKN45	-	RT-PCR Northern blot	-	Promoted colony formation and cell growth.	-	[87]
GPA34	8 (16)	5 (17)	-	-	RT-PCR, Immunohistochemistry	-	-	-	[88]
TRAG-3	Tissue: 5 (50) Cell line: 6 (9)	-	MKN7 MKN45 NS8 NUGC3 NUGC4 AZ521 KATO3 SCH GOTO	-	RT-PCR	-	-	-	[119]
BRDT	2 (10)	-	-	-	RT-PCR	-	-	-	[120]
LAGE1	17 (101)	-	-	-	RT-PCR	-	-	-	[74]
SCP1	6 (101) 24 (102)	-	-	-	RT-PCR	-	-	-	[114]
IGSF11	7 (8)	-	MKN1 MKN28 MKN45 MKN74 Kato III St-4	-	RT-PCR	-	Promoted tumor cells growth and colony formation.	-	[121]
CAGE	11 (15)	-	-	3 (36)	RT-PCR Western blot	-	-	-	[38]
XAGE1	2 (18)		SNU484		RT-PCR			Expression of XAGE1 is correlated with CpG island Hypomethylation.	[122]
ZNF165	6 (14)	-	-	-	RT-PCR	-	-	-	[123]

BJ-HCC-20	3 (14)	-	-	-	RT-PCR	-	-	-	[124]

Table 2. Summary of clinical trials being conducted in GC patients using CTAs.

	Trial status	Study title	Number of patients	Phase	Study year	Purpose and detailed description	Clinical trial identifier
1	Active, not recruiting	Multi-tumor study to assess the safety, tolerability and antitumor activity of genetically engineered MAGE-A4^{c1032}T in HLA-A2+ subjects with MAGE-A4 positive tumors	52	I	2017-2021	- Autologous genetically modified MAGE-A4c1032T cells therapy combined with low dose radiation - The participants must have some criteria: HLA-A2+, MAGE-A4 positive tumor cells and whose urinary bladder, melanoma, head and neck, ovarian, non-small cell lung, esophageal, gastric, synovial sarcoma, or myxoid/round call liposarcoma (MRCLS) tumor - Assess the safety, tolerability, and antitumor activity	**NCT03132922**
2	Recruiting participants	Assess safety and efficacy of ADP-A2M4CD8 as monotherapy or in combination with Nivolumab in HLA-A2+ subjects with MAGE-A4 positive tumors	90	I	2019-2022	- Autologous genetically modified ADP-A2M4CD8 cells alone or in combination with Nivolumab - The participants must have some criteria: HLA-A2+, MAGE-A4 positive tumor cells and whose endometrial, esophageal, esophagogastric junction, gastric, head and neck, melanoma, ovarian, non-small cell lung, urothelial cancers - Assess the safety, tolerability, and antitumor activity	**NCT04044859**
3	Recruiting participants	T cell receptor gene therapy targeting KK-LC-1 for gastric, breast, cervical, lung, and other KK-LC-1 positive epithelial cancers	100	I	2021-2022	- Determine the safety of different doses of KK-LC-1 TCR T cells plus IL-2 (Aldesleukin)/ Cyclophosphamide/ Fludarabine - Participants: patients with metastatic or refractory/recurrent KK-LC-1 positive epithelial cancer	**NCT05035407**
4	Completed	Application of NY-ESO-1-specific TCR affinity enhancing specific T cell therapy (TAEST16001) in solid tumors except non-small cell lung cancer	6	I	2017-2020	- Investigate the safety and tolerability of TAEST16001 (TCR affinity enhancing specific T cell therapy) plus chemotherapy with cyclophosphamide/fludarabine/IL-2 - The participants must have some criteria: HLA-A*0201+ and NY-ESO-1 positive cells ≥ 25% by immunohistochemistry and whose the multi-line treatment failed advanced solid tumors except non-small cell lung cancer	**NCT03159585**
5	Completed	Safety and immunogenicity of Cholesterol-Bearing Hydrophobized Pullulan HER2 Protein 146 (CHP-HER2) and NY-ESO-1 Protein (CHP-NY-ESO-1) in combination with OK-432 (Picibanil) in HER2- and/or NY-ESO-1-expressing cancers	9	I	2006-2009	- Investigate safety and immune responses including HER2 and NY-ESO-1 specific IgG and T cells - The participants must have some criteria: high risk of recurrence or metastasis and whose the esophageal, lung, gastric, breast, and ovarian cancers	**NCT00291473**
6	Completed	mTOR inhibition with Rapamycin for enhancing intranodal dendritic cell vaccine induced anti-tumor immunity in patients with NY-ESO-1	18	I	2012-2016	- DEC-205/NY-ESO-1 fusion protein CDX-1401 with and without Sirolimus - Assess the safety and toxicity	**NCT01522820**

		expressing solid tumors (vaccine therapy and immunotherapy				- Patients with any solid tumors at high risk of recurrence or with minimal residual disease	
7	Recruiting participants	EGFRvIII/DR5/NY-ESO-1/Mesothelin CAR T/TCR-T cells immunotherapy for solid malignancies	50	I/II	2019-2021	- A multi-target gene-modified immunotherapy - The participants must have some criteria: HLA-A*0201+, NY-ESO-1, Mesothelin, EGFRvIII, and DR5 positive cells by immunohistochemistry and whose the esophagus, hepatoma, glioma, and gastric cancers - Investigate safety and clinical response to T-cell infusion	**NCT03941626**

Data taken from https://clinicaltrials.gov
mTOR: Mechanistic target of rapamycin; DC: Dendritic cell; TAA: Tumor associated antigen; TCR: T-cell receptor