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REVIEW

CEA vaccines

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ABSTRACT

Carcinoembryonic antigen (CEA) is a glycosylated cell surface oncofetal protein involved in adhesion, proliferation, and migration that is highly upregulated in multiple carcinomas and has long been a promising target for cancer vaccination. This review summarizes the progress to date in the development of CEA vaccines, examining both pre-clinical and clinical studies across a variety of vaccine platforms that in aggregate, begin to reveal some critical insights. These studies demonstrate the ability of CEA vaccines to break immunologic tolerance and elicit CEA-specific immunity, which associates with improved clinical outcomes in select individuals. Approaches that have combined replicating viral vectors, with heterologous boosting and different adjuvant strategies have been particularly promising but, these early clinical trial results will require confirmatory studies. Collectively, these studies suggest that clinical efficacy likely depends upon harnessing a potent vaccine combination in an appropriate clinical setting to fully realize the potential of CEA vaccination.

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Introduction

CEA association with cancer and function

Carcinoembryonic antigen (CEA) is a 200kD glycoprotein first identified in the embryonic endodermal epithelium and was initially reported to be detectable in colon cancer cells by Gold and Freeman in 1965.[1,2] The expression of this protein is elevated in various malignancies such as colorectal and medullary thyroid cancer, and a subset of breast, mucinous ovarian, gallbladder, pancreas, gastric, and lung cancers. However, it is not a definitive marker of a particular site for cancer origin.[3,4] CEA is involved in cellular adhesion and belongs to the immunoglobulin family called CEA-related cell adhesion molecules (CEACAMs), being designated CEACAM5.[5] A variety of studies have determined that CEA can aid in the adhesion, proliferation, and migration of cells both in vivo and in vitro.[6] CEA also functions in suppressing anoikis, potentially related to its endoluminal expression and adherence function, which likely aids in tumor pathogenesis.[4,7,8] Moreover, CEA is a glycophosphatidylinositol- (GPI-) linked membrane-anchoring protein exposed to the cell surface that can be released,[9] allowing for the formation of immune complexes with induced antibodies or the ability to neutralize the efficacy of therapeutic CEA-specific antibodies.

CEA as a biomarker and target for cancer therapy

Given its elevated expression in cancer and secretion by cancer cells, CEA has been extensively investigated as a biomarker for multiple cancers, using a variety of different techniques.[10] In colorectal cancers, persistent elevation of circulating CEA after colorectal surgical resection is associated with worse survival.[11] Likewise, metastatic disease in breast and colon cancer is indicated by serum titers greater than 20 µg/L.[12] Serum CEA levels are also used to monitor for disease progression in medullary thyroid and colon carcinomas and may be used if found to be elevated in other cancers (e.g., non-small cell lung cancer).[13–15] Thus, while not specific to a particular cancer, elevated CEA expression is highly associated with tumor burden for multiple cancers and predictive of clinical outcome, thus a potent and validated tumor associated antigen (TAA) present in both primary and metastatic cancer cells.[4]

While not the focus of this review, CEA has also been targeted by immunologic but non-vaccine-based approaches. Early approaches involved radiolabeled anti-CEA antibody, but responses were limited perhaps due to anti-drug antibodies against the chimeric portion of the antibodies.[16] The antibody drug conjugate tusamitamab ravtansine (SAR408701) has reported to medate clinical responses in high expressers of CEACAM5 (CEA) in a phase I study[17] and is now in three phase III studies as monotherapy or in combination with other anti-cancer therapies in lung cancer patients.

CEA as a target for vaccines

Due to its elevated expression on the surface of multiple cancers, as well as its clinically predictive value, CEA has long been regarded as an excellent target for cancer therapies. However, as a TAA, CEA is also expressed in different normal tissues throughout the body, and thus, there may possess a significant degree of immune tolerance. Furthermore, even

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if tolerance can be overcome by potent vaccine technologies, there is a greater risk that an autoimmune adverse event might occur. Hence, the goal is to generate a relevant immune response to tumor associated CEA without inducing autoimmunity. Historically, the goal of CEA-based vaccines has been to activate and expand CD8+ CTLs against CEA-bearing cancer cells and to this end, a majority of studies have focused on CD8+ T cell responses.[18,19] Indeed, using genetically engineered murine models that express human CEA, it has been demonstrated that vaccines can overcome immune tolerance and elicit immune responses against tumors expressing CEA without causing bowel toxicity.[20,21]

Different CEA vaccine platforms

In the history of modern vaccinology, a variety of different platforms have been developed, which offer distinct advantages and disadvantages in regard to immunogenicity, off-target immune responses, safety, scalability, and ease of production. As CEA has been a vaccine target in cancer for several decades, a variety of different vaccine platforms have been employed to target this antigen, broadly including protein/peptide, DNA, viral vector, dendritic cell, and exosome-based vaccines, each with their unique mechanisms and advantages. We will describe a number of different vaccine platforms that have been employed to target CEA, as well as review the pre-clinical and clinical outcomes of these studies.

Pre-clinical CEA protein and peptide-based vaccines

Initial strategies utilized to elicit immunity against CEA involved the use of proteins and CEA-specific peptide vaccines. In an early study, Salgaller et al. utilized a baculovirus system to express a full-length human CEA (bV-CEA) protein, which was recognized by 24 different anti-CEA monoclonal antibodies.[22] Immunization with bV-CEA using an adjuvant consisting of a cationic liposome formulation (DOTAP) led to preventative anti-tumor immunity against CEA expressing colorectal cancer cells (MC38-CEA-2) in mouse models.[23] Similarly, administration of CpG-ODN with a Tat-CEA fusion protein also led to preventative anti-tumor immunity against MC38-CEA-2 tumor cells. This study also documented an increase in CEA-specific IgG2A responses, as well as CEA-specific cytotoxic T-lymphocyte response.[24] To enhance CEA protein responses, other investigators have utilized exosomal delivery from heat stressed CEA+ tumor cells, which led to enhanced DC maturation and increased CEA-specific CTL response.[25] While demonstrating the potential to elicit anti-CEA responses, the purification of proteins (or exosomal preparations) imposes limitations that may be circumvented by focusing on specific CEA peptide vaccines.

As an alternative to protein-base vaccines, CEA peptides are easier to manufacture and have a long safety record in administration, as well as an ability to specifically target immunodominant epitopes. However, this approach restricts the ability to target many epitopes, is often restricted to certain HLA haplotypes (most generally HLA-$A2^{+}$) and has often been characterized by poor immunogenicity.[26] To combat these limitations and identify immunogenic epitopes, a study conducted by Huarte et al found that selective replacements of amino acids were able to enhance the immunogenicity of CTL epitopes from CEA when used in peptide-based vaccine in HLA-A2Kb-transgenic mice.[27] In a subsequent study, immunization with an altered HLA A2 restricted peptide epitope of CEA (called CAP-1(6D) (YLSGADLNL, with Asn at position 6 replaced by Asp) was found to enhance the induction and activation of T cells that could recognize the native peptide.[22–24] This CEA peptide was thought to enhance responses by up-regulating the lymphotactin gene, thought to aid in T-cell activation.[28–31] Additionally, modified CEA agonist and antagonist peptides have also been described, composed of peptides that have mutations to enhance their immunogenicity alone or in combination with GM-CSF, IL-4, ICAM-1, LFA3, B7.1, B7.2, IFNγ, or TNFα. CEA peptide immunogenicity has also been enhanced through the conjugation to helper peptides or large carrier molecules, such as tetanus toxoid, poly-L-lysine, *Pseudomonas* exotoxin A (US Patents US20070048860, US20100209386).

Clinical CEA protein and peptide-based vaccines

Many of the earliest clinical trials of CEA vaccines, involved the use of CEA protein and peptide immunization.[32] In one early Phase I trial, recombinant baculovirus produced human CEA was administered either alone or in combination with GM-CSF to a group of 18 patients that had colorectal carcinoma (CRC) without macroscopic disease after surgery. Notably, all patients in the GM-CSF group had a strong CEA-dose-dependent IgG antibody response and a strong T cell response to CEA as compared to only three in the CEA alone group that produced a weak antibody response and a weak T cell response.[33] A dose ranging study of this approach was conducted in 24 CRC patients immunized with CEA alone or CEA with GM-CSF. This study noted the induction of strong anti-CEA specific T cell response in a group that received CEA plus GM-CSF and a strong anti- CEA IgG antibody response. However, no dose response relationship was noted with CEA protein levels. It was noted that high anti-CEA antibody titer also led to longer survival.[34] Subsequent analysis also revealed that CEA-specific IgA responses were cytotoxic and associated with a long-term survival benefit.[35] These studies have documented the ability of CEA proteins and peptides, in conjunction with different adjuvant strategies, to break tolerance and elicit CEA-specific adaptive immunity. While some studies have also suggested some survival benefits, these have been restricted to select patients in early phase trials.

DNA based CEA vaccines

As with protein-based vaccines, DNA vaccines have been tested in many settings to induce immunity against CEA. Using a transgenic mouse model tolerant to human CEA, Luo et al. illustrated that a CEA plasmid vaccine formulated into cationic microparticles with GM-CSF could break tolerance and elicit CEA-specific anti-tumor responses capable of rejecting CEA tumors in 50% of mice.[36] Another strategy utilized a plasmid encoding a truncated form of CEA fused to a tetanus toxoid epitope to improve antigen presentation

(pKCEA66), which resulted in stronger IgG1 and cell-mediated responses compared to non-modified CEA plasmid vaccination. Secondary studies confirmed these findings and also demonstrated that boosting plasmid CEA vaccines with recombinant CEA immunizations induced a significant increase in humoral anti-CEA Ab response in mice.[37,38] These studies also demonstrated that adaptive anti-CEA responses were capable of protecting mice against CEA tumor growth in preventative vaccine settings.[37] In another study a plasmid DNA vaccine was designed that expressed a triple repeated CEA peptide that was capable of eliciting significant induction of anti-CEA antibodies were observed.[39] Other approaches to enhance plasmid vaccine immunogenicity have utilized co-injection of plasmids encoding soluble B7.1/IgG Fc fusion protein to induce more potent CEA-specific immunity.[40] This approach was found to augment CEA plasmid vaccination to enhance the induction of CD8+ T cells and protect against CEA+ cancers.[41] Parallel approaches to enhance CEA-specific responses have used co-expression from plasmids of strong adjuvant cytokines. Most notably, co-expression of IL-12 from plasmid vaccination has been demonstrated to elicit stronger anti-tumor effects and protection from CEA+ lung cancer cells, as well as greater CEA-specific antibody and T cell responses. In this study, intramuscular injection of these plasmids led to 80% protection of mice against CEA+ tumor challenge.[42] Other studies have used co-expression of GM-CSF or recombinant GM-CSF with CEA, demonstrating that this combination produced better cellular and humoral responses than mice immunized with CEA-DNA alone, as well as protection against MC38-CEA-2 cells.[40] Finally, an advantage to plasmid is the ability to utilize homologous boosting strategies as another means to enhance responses. In one study, intradermal immunization using a CEA expressing plasmid was significantly enhanced by homologous late boosting using electroporation of a CEA plasmid, leading to a 10-fold increase in $CD8^+$ T cells and enhanced CEA-specific IgG responses compared to CEA protein boosting strategies.[43] Additionally, in studies using targeted therapies such as Sunitinib, a multitargeted receptor tyrosine kinase inhibitor that decreases T-regs and MDSCs and increase INFγ-producing T cells it was found that combination Sunitinib with a viral vaccine encoding CEA decreased the tumor volume in a mouse model.[44,45]

These CEA encoding DNA vaccines were delivered by intramuscular or intradermal injection of large quantities of CEA plasmid. In contrast, some studies have utilized oral immunization of CEA DNA plasmids using a Salmonella typhimurium (SL7207) bacteria carrier. Using transgenic CEA mice, investigators have demonstrated that oral administration of a CEA vaccine (fused with human CD40L) in Salmonella was effective in breaking peripheral T cell tolerance against human CEA and capable of inducing tumor protective immunity against MC38-CEA tumor challenge, associated with activation of CD8 T cells and DCs.[46,47] The use of Salmonella-CEA DNA oral vaccination also demonstrated anti-tumor protection against pulmonary CEA+ metastases.[47] Additionally, to improve efficacy of oral vaccines, researchers have combined fusion protein of human IL-2 and Fc fragment of human IgG1 with oral CEA-based DNA vaccines.[48,49] These studies utilized boosting with a fusion IL-2 protein to augment T cell responses and enhance anti-tumor immunity against CEA. Collectively, these studies have documented the potential of CEA plasmid vaccines to elicit immunity, although they have required additional adjuvant strategies and have typically been found to elicit preventative immunity against CEA+ cancer, instead of therapeutic treatment against established CEA+ tumors.

Clinical studies of DNA-based and RNA-based CEA vaccines

In 2002, a Phase I clinical trial evaluated the safety and immunogenicity of a DNA vaccine construct consisting of CEA and hepatitis B surface antigen in 17 patients with metastatic colorectal cancer. While the vaccine was well tolerated, 12 patients had progressive disease and five had stable disease. CEA-specific antibodies were not observed, but lymphoproliferative responses to CEA were detected in 4 of 17 patients.[50] Based on previous pre-clinical studies using pKCEA66 (truncated CEA fused with tetanus toxoid), a phase I trial was conducted in 10 post-resection colorectal cancer patients, which had stage II or III cancers. In this study, patients were divided into two groups, receiving three cycles of intradermal or intramuscular injections of pKCEA66 after receiving systemic chemotherapy and GM-CSF treatments. These vaccines were well tolerated and encouragingly, 8/10 patients had no evidence of disease upon follow-up.[51–53] In a similar approach, CEA mRNA was produced from plasmids encoding multiple tumor associated antigens (MUC1, CEA, Her-2/neu, telomerase, survivin, and MAGE-A1) and injected with GM-CSF in a phase I-II study in renal carcinoma patients. This study tested two vaccine doses (20ug and 50ug) in 30 patients, finding that 6 patients in one group and 9 in the higher dose group had stable disease with induction of CD4+ and CD8+ T cell responses.[54] In an exploratory phase I/II trial of an anti-CEA DNA fusion-vaccine encoding pDOM-CAP-1 in patients with CEA-expressing cancers it was found that the vaccine was well tolerated and there was an 86% reduction in risk of death.[55] In another study of vectored DNA-based vaccines it was found that GI-6207, a promising agent based on a heat-killed *Saccharomyces cerevisiae* strain genetically modified to express CEA was determined to be safe and displayed a degree of efficacy in patients with CEA-expressing carcinomas.[56]

Viral CEA vaccine platforms

In contrast to protein, peptide, and DNA/RNA vaccines, a large variety of different viral vector vaccines have been employed in developing and testing CEA vaccines. In general, these viral vectors capitalize on the natural infection machinery of viruses to deliver antigens and stimulate robust immune responses, but may have limitations in eliciting off-target vector-specific effects. This may include a more limited ability to use homologous boosting to amplify CEA-specific responses, as well as be less amenable to scaling for clinical manufacture. However, many of these vectors have been successfully used in

the clinical and offer strong potential to elicit CEA-specific adaptive immune responses.

Vaccinia backbone CEA vaccines

In 1992, one of the first viral vector CEA vaccines was developed using a modified vaccinia backbone (New York strain), which had been used as a smallpox vaccine in humans. Vaccination with rV (NYC)-CEA was found to protect against CEA tumor implantation and suppress the growth of established CEA expressing tumors in mice.[57] This vaccine was also found to elicit responses against CEA in nonhuman primates, as well as demonstrate an acceptable safety profile. Moreover, these vectors have also been utilized to generate CEA cancer cell vaccines, which proved to be more immune-stimulatory in comparison to retroviral vectors.[58,59] These studies led to a Phase I clinical trial I 1995 using a recombinant vaccinia-CEA in metastatic cancer patients.[60] While no clinical anti-tumor effects were reported, cytolytic CEA-specific T cells were expanded after vaccination in contrast to an inability to expand these cells in pre-immunized PBMCs, suggesting that vaccination was able to overcome some forms of immune tolerance to expand CEA-specific T cell populations in patients.[60]

ALVAC backbone CEA vaccines

A potential drawback in using vaccinia vectors is their documented immunogenicity which limits their serial boosting capacity. In contrast, the attenuated canary pox recombinant vector, ALVAC, only replicates in avian cells and expresses transgene products for 14 to 21 d, without infecting other cells.[18,19,59] In 1997, an ALVAC-CEA vector was developed and demonstrated to have antitumor activity in immune competent mice.[61] Based on these results, a Phase I dose escalation trial of ALVAC-CEA was initiated of patients with advanced cancers with CEA serum levels >10 ng/ml or having a positive CEA tumor by IHC. The study incorporated three ALVAC-CEA injection (spaced 4 weeks apart) and did not record any adverse toxicities.[18] While only one patient displayed normalization of CEA levels without disease progression for 15 months, all patients (regardless of dose level) showed increased CTL precursor frequencies. As this trial demonstrated safety but did not demonstrate significant antitumor activity, a dual gene vector ALVAC (expressing CEA and B7.1) was developed to enhance anti-tumor responses. This vaccine was used in a clinical study of 39 patients with advanced or metastatic CEA+ carcinomas.[59] These patients all received four total intradermal ALVAC-CEA-B7.1 injection of 4.5×10^8 pfu (every 2 weeks) with stable disease patients (at 8 weeks) given boosts at 4 weeks. Patients with stable disease at 8 weeks were given boost injections every 4 weeks and reevaluated subsequently every 8 weeks. The treatment did not elicit adverse events and 8 of 30 (~27%) patients had stable disease, while 17 of 30 (~57%) patients had declines in serum CEA. However, the majority of patients eventually experienced rising serum CEA levels and immunologic responses were found to be less likely in patients with chemotherapy pretreatment, suggesting that vaccine needed to be used in patients with minimal disease.[59]

Adenoviral backbone CEA vaccines

Other well-developed viral vaccine systems are based on adenoviral vectors, which allow for strong transgene expression with immune stimulation to target different cancer antigens. In early studies, 1st generation [E1, E3-] adenoviral serotype 5 (Ad5) vectors were developed and demonstrated the ability to simulate immunity against CEA in mouse models.[62,63] Given the ubiquitous nature of immunity against this serotype in humans, other groups have developed CEA adenoviral vectors based on a chimpanzee serotype 3,[64] as well as 2nd generation adenoviral vectors [E1, E2b-, E3-] that permit the induction of CEA immunity, despite the presence of preexisting Ad5 immunity.[65] This vector was utilized in an escalating dose Phase I/II clinical trial where CEA-specific T cell responses were induced in the majority of patients (~61%), without an impact from preexisting immunity against Ad5.[66] As another means to enhance CEA-specific immune responses, adenoviral CEA expression was modified by fusion to exosomal targeting sequences to traffic CEA to lysosomes, which resulted in enhanced CEA-specific T cell responses in pre-clinical models.[67] Other approaches have used CEA fusion with the minimized domain of tetanus toxin fragment C (Ad/CEA-DOM) or the B subunit of the heat-labile enterotoxin (Ad/CEA-LTB) to enhance CEA-specific CTL responses and anti-tumor action after adenoviral vaccination.[68,69] Finally, other studies have combined Ad-CEA vaccines with anti-PD1 immune checkpoint inhibitors, suggesting a potential clinical path forward in the use of CEA vaccines in advanced immunosuppressive cancers.[70]

Adeno-associated viral vector vaccines

Recombinant adeno-associated virus vector (rAAV) may be a promising vaccine vector as compared to adenoviral, lentiviral, and retroviral vectors due to rAAV not integrating into host genome and its anti-oncogenic properties against human papillomavirus (HPV)-induced carcinogenesis.[71–73] In one study, effective delivery of CEA through rAAV with GM-CSF adjuvant helped improve immune infiltrates into vaccine site and helped provide antigen-specific anti-tumor response.[5] In another study, co-administration of TLR agonists and AAV2 encoding CEA led to antitumor response against MC38 cells in mice with colon cancer.[74] In a phase I trial, patients were administered CTLs that had been activated by DCs that had previously been transduced with AAV2 vectors carrying CEA. These patients had failed to response to standard treatment. In the 25 patients, 2 showed partial remission, 10 showed stable diseases, and 13 had progressive disease, with a resulting mean progression-free survival of 3.1 months.[75]

Anti-idiotype antibody-based vaccines

Anti-idiotype antibodies have also been used to induce TAA-specific humoral responses. In one study a monoclonal anti-Id antibody named 3H1 that mimics a specific epitope of human CEA was developed. When used as a tumor vaccine against CEA in murine cancer models it showed high titers of anti-CEA antibodies and protection of mice against MC38-CEA cells. Additionally, when CpG oligodeoxynucleotides (CpG-ODN) were used as an adjuvant, stronger anti-tumor responses were noted.[76–78]

In another study, a mouse anti-Id antibody was produced that mimics CEA. While this antibody did not display striking immunogenicity, modified of this antibody (patent: US200502– 22392) allowed for strong induction of CEA-specific immunity[79] Additionally, a fusion protein composed of a single chain variable fragment of an anti-Id antibody mimicking an epitope specific for human CEA (scFv6G6.C4) and mGMCSF were observed to overcome tolerance in CEA-transgenic mice. In C57BL/6 mice that were immunized with fusion protein there was a stronger anti-CEA antibody response to both scFv6G6.C4 and CEA than mice immunized with scFv6G6.C4 alone.[80] In a similar manner another group developed an anti-Id scFv isolated from an anti-Id monoclonal antibody (6.C4) that mimicked CEA. This scFv mimicked CEA and was able to stimulate a humoral response against CEA in BALB/c mice.[81] In another study when Tetanus Toxin Fragment C (FrC) was added as an adjuvant, it led to prevention of tumor growth in mice challenged by MC38-CEA cells.[82] The same group also tested IFN-γ and GM-CSF were as adjuvants. Addition of IFN-γ led to higher CD4+ and CD8+ responses and 80% of mice immunized with vaccine plus adjuvant did not develop tumors or delayed growth of tumors.[83] In another study, scFv6.C4 expressing plasmid vector (uP/PS-scFv6.C4) was used in mice expressing CEA coupled with electroporation. Mice immunized with the uP empty vector died within 40 d, but uP/PS-scFv6.C4 vaccinated mice (40%) remained free of tumor for more than 100 d.[84]

Self-replicating RNA CEA vaccines

Another vector system that promises to enhance immune responses through a combination of replication and homologous boosting potential is that of self-replicating RNA vaccines.[85] In this platform, positive strand RNA viruses act as the template where the structural protein genes are replaced with genes of interest, while structural proteins provided in *trans* (not packaged into the replicon) create single cycle viral replicon particles (VRPs). Alphaviruses such as Venezuelan Equine Encephalitis (VEE) serve as the vector for these VRPs.[85] Using a VRP-CEA, a dose escalation phase I clinical trial was performed in 28 patients with advanced CEA+ cancers (Stage III and IV) that demonstrated an ability to induce clinically relevant CEA-specific T cell and antibody responses, in spite of elevated levels of T regulatory cells.[86] Notably, long-term follow-up of patients vaccinated with these vectors (median time >10 y) revealed that all patients demonstrated the presence of CEA-specific humoral immunity with 10/12 (with Stage III CEA+ cancers) having an increase in CD8+ effector memory T cell responses. While ~17% of Stage IV CEA+ patients were alive at 5 y, all patients with Stage III cancer were alive. While a limited number of patients, CEA-specific vaccination demonstrated some potential clinical benefit, especially in advanced cancers that have not metastasized. To potentially enhance these responses, IL-12 was included in VRP-CEA vaccines, which were tested pre-clinically to assess the potential anti-tumor effect.[87] These studies demonstrated the induction of CEA-specific immunity was enhanced by local IL-12 expression, as well as anti-tumor efficacy.

CEA vaccine combinations

Given the lack of efficacy with single vaccinations, heterologous boosting using different backbone CEA vaccines has also been tested. In a pre-clinical study demonstrating heterologous boosting, a CEA expressing DNA prime vaccine was combined with an adenoviral-boosting vaccine to document enhanced immunity against CEA+ prostate cancer cell challenge, with reduced tumor growth and prolonged survival compared to immunization with a single modality.[88] In another study, a rV-CEA viral vaccine prime was followed by CEA protein immunization that led to enhanced immunity and anti-tumor activity in mice, greater than either vaccine alone.[64] Many other approaches have used heterologous vaccine combinations to stimulate CEA immunity. In one early study, an rV-CEA priming vaccine was successfully boosted by ALVAC-CEA to elicit a nearly 4 times greater CEA-specific lymphoproliferation response and anti-tumor responses, against MC38-CEA tumor challenges in mice.[61] This approach was subsequently employed clinically in a Phase I trial of 18 patients with CEA+ carcinomas.[89] In this trail, all patients received a priming dose of rV-CEA with half patients receiving boosting doses (3 immunizations at 4-week intervals) of ALVAC-CEA. No toxicities were observed and greater induction of CEA-specific T cell frequencies were observed in the boosting group, although no objective antitumor responses were observed.[89] This trial was then expanded into a Phase I/II trial by adding GM-CSF and IL-2 to the vaccine combination.[90] While only nine patients were enrolled, those that received GM-CSF (but not GM-CSF +IL-2) demonstrated significantly higher T cell counts in comparisons to those that had also received IL-2.[90] To further improve responses, additional immune stimulatory genes were incorporated into these vaccines, such as B7.1, ICAM-1, and LFA-3 (known as TRICOM) to enhance T cell co-stimulation.[91] Pre-clinical studies using rV-CEA-TRICOM and a replication-defective avipox (rF-CEA-TRICOM) found that TRICOM vector elicited enhanced CEA-specific T cell responses, while the addition of GM-CSF and IL-2 enhanced anti-tumor efficacy in pre-clinical models.[92] Based on these studies, a Phase I clinical trial was initiated using rV-CEA-TRICOM and rF-CEA-TRICOM vectors in 56 patients with a variety of CEA+ tumors. Patients were randomized to eight different groups to assess different vector boosting combinations with or without GM-CSF. The vaccines were well tolerated, although one patient experienced abdominal pain that required hospitalization. A majority of patients had CEA-specific T cell responses

over baseline, although several had evidence of these responses prior to vaccination. Notably, in 40% of patients had stable disease with ~ 25% having prolonged stable disease (>6 months) and 1 patient having a complete pathologic response.[93] In another phase I clinical trial evaluating the combination of GM-CSF and IFN-α with the novel anti-CEA vaccines (rV-CEA(6D)-TRICOM and rF-CEA(6D)-TRICOM) it was found that stable disease was observed in 24% of patients and no partial responses. Overall survival was significantly increased with the addition of IFN-α-2b to the treatment regimen.[94] Other studies have included additional targets in combination with CEA, such as mucin-1 (MUC-1), in identical rV/rF-CEA/MUC-1 vector[95] to improve anti-tumor immune targeting or have incorporated Brachyury and MUC-1 into vaccines, alongside CEA.[96,97] Likewise, standard-of-care modalities such as chemotherapy or T cell augmenting strategies, such as PD-1/PD-L1 blockade have also been utilized to enhance CEA vaccine efficacy in early clinical trials.[98] In a study conducted in metastatic colorectal cancer (mCRC) patients with MMR proficient tumors that do not respond to immune checkpoint inhibition, it was found that a combination of avelumab (Av) + CEA-targeted adenoviral vaccine (Ad5) + standard of care (SOC) mFOLFOX6 + bevacizumab had synergistic anti-tumor activity.[98,99]

Dendritic cell vaccines

A different CEA vaccine strategy has employed ex vivo-generated dendritic cells (DCs), professional antigen presenting cells, loaded with CEA before re-infusion as a cellular vaccine. In early studies, DCs were pulsed with a CEA-Hsp70-like protein 1 (CEA-Hsp70L1) fusion protein ex vivo which promoted DC maturation and induction of CTLs after infusion in to mice.[100–102] Other studies have delivered mRNA into DCs encoding a different CEA fusion protein (CRT-TAT-CEA) to enhance CEA-specific immunity.[103] Immunization with these DCs enhanced CD4 and CD8 T cell responses and suppressed MC38-CEA engraftment and tumor growth.[104] CEA mRNA transduction of DCs was utilized in a Phase II study that demonstrated the induction of CEA-specific immunity, although progression of disease occurred in the majority of patients.[105] CEA epitopes were also used to pulse DCs in combination in combination with a monoclonal antibody 3H1 to improve CD4+ T-helper and CTL responses against MC38-CEA tumor cells.[106] In addition to protein, peptide and mRNA pulsing strategies, viral vectors have also been used to transduce DCs ex vivo as a cellular vaccine. In initial studies, DCs were transduced with recombinant adenoviral vectors (Ad-CEA), which led to activation of CEA-specific T cells and suppressed MC38-CEA tumor growth.[107] Improving upon this strategy, Ad vectors encoding a combination of CEA, GM-CSF, and IL-12 or survivin (SVV) also generated potent CEA-specific immunity that translated into significantly more impactful anti-tumor responses.[108] Clinical studies have also utilized DCs transduced with different viral vectors. Using rV-CEA, DCs were transduced and delivered in combination with a Treg depletion strategy in a Phase II clinical trial that demonstrated enhanced CEA-specific immunity in combination treatment groups.[109] In another study using mouse induced pluripotent stem cell (iPSC)-derived dendritic cells (miPSDCs) transduced with full-length CEA cDNA it was observed that there was strong cytotoxic activity against CEA-positive target cells in a CEA transgenic mouse model. This strategy was observed to overcome the weaknesses of previous DC-based vaccine strategies with regard to sufficient numbers.[110] While DC vaccines have further demonstrated the potential of CEA-based vaccines, the challenge of producing personalized DCs in a clinical setting may limit their widespread utilization, especially in light of more robust RNA-based vaccine platforms.

Nanoparticle based delivery system of vaccines

Nanoparticles have the ability to modulate immune responses and heighten protective immunity and hence represent a promising adjuvant as well as a delivery system.[111–113] Similarly, exosomes which are a class of bi-layered membrane vesicles that are present in blood, saliva, and breast milk can be used as vaccine candidates due to their stability, vascular permeability, biodistribution, and solubility.[114] In colorectal cancer patients it has been speculated that extraction of exosomes from effusions and activation of immune responses by exosomes produced in ascites (Aex) might be a source of therapeutic intervention. Aex has been observed to activate CD8+ CTLs and possibly induce antitumor immunity to CEA. Exosomes generated by heat-stressed CEA-positive tumor cells were found to initiate and increase an HLA-A *0201-restricted and CEA-specific CTL response.[115] Additionally, when Aex was co-administered with GM-CSF better CEA-specific CTL responses and HLA-A *0201 restriction was observed as compared to Aex alone.[116,117]

Discussion

To date, there have been no therapeutic cancer vaccines targeting CEA that have received US marketing approval from the FDA. For example, a Phase III trial investigating PANVAC-CEA in pancreatic cancer was initiated in 2006 (NCT00088660), but this trial did not reach its endpoint and its results remain unpublished.[118,119] These comprehensive investigations (summarized in Tables 1 and 2), spanning both pre-clinical and clinical realms, have explored diverse modalities and combinations in the realm of CEA vaccines. They also reveal the complexities of CEA as a vaccine target. Although overexpressed on tumors, CEA is a self-antigen to which there could be tolerance; however, numerous clinical studies have demonstrated that tolerance to this antigen can be broken, albeit the magnitude of these responses may be suboptimal.

As a cell surface antigen, CEA can be targeted by both T cell and B cell responses, although its secretion could mute immunologic responses elicited by antibody targeting. Importantly, although various studies have documented a contribution from CEA in multiple biological processes, its precise role and importance in cancer continues to lack clarity. If the CEA contribution to cancer is limited, it suggests that successful CEA vaccines could lead to immune editing without a significant clinical benefit. On the contrary, if CEA significantly influences cancer development, effective CEA vaccines could become pivotal in triggering a robust

Table 1. CEA vaccine studies in mice.

	Mouse Model	Effector cells	Effect on mice	Ref
Protein based vaccines-preclinical trials				
bV-CEA/DOTAP	MC38-CEA-2 cells in C57BL/6 mice	Antibodies and T-cells	Protection from tumor challenge (70% of mice)	23
CpG-ODN plus Tat-CEA fusion protein	MC38-CEA-2 cells in C57BL/6 mice	CTLs and IgG	Prolongation of survival time (70% of mice), reduction of tumor volume (100% of mice)	24
CEA+/HS-Exo	HLA-A2.1/Kb transgenic mice	CTLs	No effect	25
DNA based vaccines alone-preclinical trials				
Prime (hCEA/DNA)-boost (GM-CSF/DNA)	MC38-CEA in CEA-tg C57BL/6 mice	CTLs	Reduced tumor volume (50%)	36
hCEA/DNA (pKCEA66 plasmid encoding a truncated hCEA form)	SCID mice with spleen cells from immunized d C57BL/6 mice and inoculated with hCEA expressing cells.	IgG, IFN-γ, T cell responses, NK cells	Suppression of tumor growth (60%)	37,120
hCEA/DNA (pCD40LT-CEA)	MC38-CEA in CEA-tg C57BL/6J mice	CTLs and DCs	Rejection of tumor challenge (50%)	46
hCEA/DNA (pCMV-CEA) plus IL-12/DNA (VR-IL-12)	CEA/LLC* cells in C57BL/6 mice	Antibodies, CTLs and IFN-γ	Suppression of tumor growth (80%)	42
hCEA/DNA (pCMV-CEA) plus IL-12/DNA (VR-IL -12) membrane bound	CEA/LLC* cells in C57BL/6 mice	Antibodies, CTLs and IFN-γ	Reduction of tumor incidence (40%)	121
Recombinant vector-based vaccines-preclinical trials				
ALVAC-CEA	MC38-CEA cells in C57BL/6 mice	CD4+ T cells, IgG, IFN-γ,IL-5	Suppression of tumor growth (100%)	122
Ad5 [E1-, E2b-]-CEA	MC38-CEA-2 cells in C57BL/6 mice	IFN-γ and IL-2	Regression of tumor growth (100% of mice)	65
Prime (rV/-CEA/TRICOM)-boost (yeast-CEA)	LL2-CEA tumor cells in CEA tg mice	CTLs	Reduction in pulmonary metastasis	123
rV-CEA/TRICOM plus GM-CSF and IL-2	MC38-CEA cells in CEA tg mice	CTLs	Prolongation of survival time (83% of mice)	92

Table 2. CEA vaccine studies in humans.

	Phase of study	Number of patients and cancer type	Effect on humans	Ref
DNA based vaccines alone+immunostimulants-clinical trials				
Single/repetitive administration of CEA DNA vaccine	I	17 patients with Stage IV colorectal cancer	CEA lymphoproliferative responses (4/17 patients)	50
CEA66 DNA immunization	I	10 patients with Stage II-III colorectal cancer	No clinical signs of autoimmunity	53
MRNA encoding MUC1, CEA, Her-2/neu, telomerase, survivin, MAGE-A1	I and II	Phase I study of 14 patients and Phase II study of 16 patients with Stage IV renal carcinoma	CD4+ and CD8+ T cell responses	54
Protein and peptide-based vaccines-clinical trials				
Multi-antigen mixed vaccine plus GM-CSF and IL-2	II	42 patients with Stage IV Breast cancer	Significant increase of lymphocyte proliferative responses	124
rCEA alone or associated with GM-CSF	I	24 patients with Stage I-II-III colorectal cancer	Long lasting anti-CEA specific T cell and IgG antibody response in GM-CSF group	34
Combined vaccination				
PANVAC-V followed by 3 administrations with PANVAC-F	I	10 patients with Stage IV Pancreatic cancer	Antibody responses against vaccinia virus in all patients	95
Recombinant Vector-based vaccines				
ALVAC-CEA	I	8 patients with Stage IV colon carcinoma	CTL responses to CEA	125
ALVAC-CEA B7.1 alone or with GM-CSF	I	30 patients with Stage IV Colorectal, breast, pancreas, appendix, esophagus, gallbladder, lung, thyroid cancer	Increases CEA-specific T cell response increase in vaccine alone group	59
ALVAC-CEA-B7.1	I	18 patients with Stage IV CEA-expressing adenocarcinomas	CEA-specific precursor T cells increase (3 SD patients)	126
CEA/TRICOM	I	12 patients with Stage I gastrointestinal cancer	No post-treatment increases in CEA-specific T cells	127
MV-CEA	I	21 patients with Stage IV ovarian cancer	Dose-dependent CEA elevation in peritoneal fluid and serum	128
rV-CEA(6D)-TRICOM	I	58 patients with Stage IV Colorectal, lung, breast, thyroid, unknown primary, ovary, gastrointestinal	Enhanced CEA-specific T cell responses in most of the patients	93
DC based vaccines				
CAP-1 pulsed PBL	I	19 patients with stage IV colorectal, breast, ovarian, pancreatic cancer	Pleomorphic infiltrates in DC injection sites (3 patients)	101
CAP1-6D DCs	I	12 patients with stage IV or recurrent colorectal and lung cancer	CD4+ and CD8+ T lymphocyte responses	129

anti-tumor immune response. While investigations of CEA vaccines have yet to result in FDA approvals, they have yielded valuable and pivotal insights that may pave the way for future progress in CEA vaccines and cancer vaccines in general (Tables 1 and 2).

One salient insight is the profound difference in various vaccine platforms ability to elicit CEA-specific immune responses, with many vaccines requiring additional boosting or immune modulation. While there have been few head-to-head comparison studies, the ability of protein and peptide

vaccines to elicit CEA-specific T cell and B cell responses is more modest in comparison to many viral vectors, while DNA-based vaccines have also been suboptimal in eliciting adaptive immunity. Of the different viral platforms, those that can replicate or be boosted appear better able to elicit CEA-specific immunity and anti-tumor efficacy, both in pre-clinical models and in more challenging clinical settings.

A second critical insight, is in the ability of optimized CEA vaccine strategies to break immune tolerance. There is much debate surrounding the antigen-specific nature of successful immunity against cancer, particularly that involving CD8+ T cell responses and the use of PD-1/PD-L1 immune checkpoint inhibition. Some evidence suggests that many of these responses are to mutated neoantigens, while other evidence suggests the primacy of responses to non-mutated antigens. While research on CEA vaccination hardly resolves this debate, it does demonstrate that immunity can be elicited and detected against self-antigens, which can associate with anti-tumor responses. Critically, there has been no evidence of profound induction of autoimmunity in the setting of vaccination against a self-antigen, thus mitigating safety concerns that surround the use of vaccines with TAAs. Both pre-clinical and clinical studies have yet to identify any evidence of toxicity or autoimmunity, which mirrors some pre-clinical studies that effective immunity against non-mutated tumor antigens may be possible without eliciting adverse pathologies. However, the role of post-translational modifications in antigen targeting remains unknown, which may allow for more tumor-selective immunity without engagement of nonmalignant tissues for identical antigens that are differentially modified.

A third insight is the ability of heterologous vaccination and additional immune modulation to enhance cancer vaccines. This can take many forms, such as the co-expression of cytokines, alteration of CEA antigens, or co-expression of immune modulators that all allow for more effective induction of CEA-specific immunity. This will be essential in counteracting the immune suppressive microenvironment encountered in advanced cancers and be beneficial in cancer vaccines, provided these modulations do not trigger adverse immune reactions.

Finally, these studies suggest that the advanced or early state of cancer may have a critical impact in the efficacy of CEA vaccination and cancer vaccines in general. Multiple pre-clinical studies have demonstrated the potent impact of CEA vaccines in preventing the implantation of CEA+ cancers, but have revealed that they are less effective against established cancers. Likewise, clinical studies in metastatic and advanced CEA+ malignancies have revealed improved outcomes in settings where tumors are resected and disease burden is minimal, likely reflecting a less advanced immune suppressive state as well as offering a smaller cancer cell population that may be more sensitive to immune pressures elicited by vaccination. This suggests that future cancer vaccine strategies may be employed after surgical resection to prevent the outgrowth of cancer or in patients at a higher risk of relapse in combination with maintenance therapies.

While effective CEA vaccines are not a current reality, the broad body of work on immunologically targeting CEA has led to critical insights in how to improve CEA vaccines, as well as new insights into oncoimmunology. The recent success of vaccines in taming the SAR-CoV-2 and development of new modalities promises further approaches that may build upon previous efforts to produce more effective CEA vaccines. As CEA is widely expressed across different cancers, highly expressed on tumors, can be targeted by both T cell and B cells, as well as easily measured, it remains a compelling clinical target for vaccination. Critically, past studies have documented an ability of CEA vaccination to break tolerance against this target and elicit anti-tumor responses in tolerant settings. Thus, while CEA vaccines are unlikely to become a stand-alone therapy, a combination of CEA vaccination with appropriate adjuvant strategies, especially those using immune checkpoint blockcade, have the strong potential to allow for the elimination of cancer recurrence. These strategies may also provide an immunologic buttress for standard therapeutic approaches, thus engaging and involving immunity against cancers more effectively than current immune checkpoint blockade strategies.

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References

1. Duffy MJ. Carcinoembryonic antigen as a marker for colorectal cancer: is it clinically useful? Clin Chem. 2001;47(4):624–30. doi:10.1093/clinchem/47.4.624.
2. Gold P, Freedman SO. Specific carcinoembryonic antigens of the human digestive system. J Exp Med. 1965;122(3):467–81. doi:10.1084/jem.122.3.467.
3. Hall C, Clarke L, Pal A, Buchwald P, Eglinton T, Wakeman C, Frizelle F. A review of the role of carcinoembryonic antigen in clinical practice. Ann Coloproctol. 2019;35(6):294–305. doi:10.3393/ac.2019.11.13.
4. Kankanala VL, Mukkamalla SKR Carcinoembryonic antigen. StatPearls. Treasure Island (FL): StatPearls Publishing. Copyright © 2023, StatPearls Publishing LLC.; 2023.
5. Hensel JA, Khattar V, Ashton R, Ponnazhagan S. Recombinant AAV-CEA tumor vaccine in combination with an immune adjuvant breaks tolerance and provides protective immunity. Mol Ther Oncolytics. 2019;12:41–8. doi:10.1016/j.omto.2018.12.004.
6. Zi-Yang Y, Kaixun Z, Dongling L, Zhou Y, Chengbin Z, Jimei C, Caojin Z. Carcinoembryonic antigen levels are increased with pulmonary output in pulmonary hypertension due to congenital heart disease. J Int Med Res. 2020;48(11):300060520964378. doi:10.1177/0300060520964378.
7. Wirth T, Soeth E, Czubayko F, Juhl H. Inhibition of endogenous carcinoembryonic antigen (CEA) increases the apoptotic rate of colon cancer cells and inhibits metastatic tumor growth. Clin Exp Metastasis. 2002;19(2):155–60. doi:10.1023/A:1014566127493.

8. Ordoñez C, Screaton RA, Ilantzis C, Stanners CP. Human carcinoembryonic antigen functions as a general inhibitor of anoikis1. Cancer Res. 2000;60:3419–24.
9. Yamamoto Y, Hirakawa E, Mori S, Hamada Y, Kawaguchi N, Matsuura N. Cleavage of carcinoembryonic antigen induces metastatic potential in colorectal carcinoma. Biochem Biophys Res Commun. 2005;333(1):223–9. doi:10.1016/j.bbrc.2005.05.084.
10. Hammarstrom S, Shively JE, Paxton RJ, Beatty BG, Larsson A, Ghosh R, Bormer O, Buchegger F, Mach JP, Burtin P, et al. Antigenic sites in carcinoembryonic antigen. Cancer Res. 1989;49 (17):4852–8.
11. Muñoz-Montaño WR, López-Basave HN, Castillo-Morales A, Castillo-Morales C, Sánchez-Trejo K, Catalán R, Díaz-Romero C, Lino-Silva LS, Maliachi-Díaz A, Ruiz-García E, et al. Persistent high levels of carcinoembryonic antigen after tumor resection are associated with poorer survival outcomes in patients with resected colon cancer. BMC Cancer. 2023;23(1):678. doi:10.1186/s12885-023-11126-4.
12. Nicholson BD, Shinkins B, Pathiraja I, Roberts NW, James TJ, Mallett S, Perera R, Primrose JN, Mant D. Blood CEA levels for detecting recurrent colorectal cancer. Cochrane Database Syst Rev. 2015;2015(12):Cd011134. doi:10.1002/14651858.CD011134.pub2.
13. Nien FJ, Chang TC. Biomarkers of medullary thyroid cancer in the prediction of cure after thyroidectomy. J Formos Med Assoc. 2015;114(9):793–4. doi:10.1016/j.jfma.2013.06.016.
14. Nasralla A, Lee J, Dang J, Turner S. Elevated preoperative CEA is associated with subclinical nodal involvement and worse survival in stage I non-small cell lung cancer: a systematic review and meta-analysis. J Cardiothorac Surg. 2020;15(1):318. doi:10.1186/s13019-020-01353-2.
15. Kuo YS, Zheng MY, Huang MF, Miao CC, Yang LH, Huang TW, Chou Y-T. Association of divergent carcinoembryonic antigen patterns and lung cancer progression. Sci Rep. 2020;10(1):2066. doi:10.1038/s41598-020-59031-1.
16. Sultana A, Shore S, Raraty MG, Vinjamuri S, Evans JE, Smith CT, Lane S, Chauhan S, Bosonnet L, Garvey C, et al. Randomised phase I/II trial assessing the safety and efficacy of radiolabelled anti-carcinoembryonic antigen I(131) KAb201 antibodies given intra-arterially or intravenously in patients with unresectable pancreatic adenocarcinoma. BMC Cancer. 2009;9:66. doi:10.1186/1471-2407-9-66.
17. Gazzah A, Bedard PL, Hierro C, Kang YK, Abdul Razak A, Ryu MH, Demers B, Fagniez N, Henry C, Hospitel M, et al. Safety, pharmacokinetics, and antitumor activity of the anti-CEACAM5-DM4 antibody–drug conjugate tusamitamab ravtansine (SAR408701) in patients with advanced solid tumors: first-in-human dose-escalation study. Ann Oncol. 2022;33(4):416–25. doi:10.1016/j.annonc.2021.12.012.
18. Marshall JL, Hawkins MJ, Tsang KY, Richmond E, Pedicano JE, Zhu MZ, Schlom J. Phase I study in cancer patients of a replication-defective avipox recombinant vaccine that expresses human carcinoembryonic antigen. J Clin Oncol. 1999;17 (1):332–7. doi:10.1200/JCO.1999.17.1.332.
19. Marshall J. Carcinoembryonic antigen-based vaccines. Semin Oncol. 2003;30(3 Suppl 8):30–6. doi:10.1016/S0093-7754(03)00233-1.
20. Bhattacharya-Chatterjee M, Saha A, Foon KA, Chatterjee SK. Carcinoembryonic antigen transgenic mouse models for immunotherapy and development of cancer vaccines. Curr Protoc Immunol. 2008;Chapter 20:20.8.1–.8.12. doi:10.1002/0471142735.im2008s80.
21. Mori F, Giannetti P, Peruzzi D, Lazzaro D, Giampaoli S, Kaufman HL, Ciliberto G, La Monica N, Aurisicchio L. A therapeutic cancer vaccine targeting carcinoembryonic antigen in intestinal carcinomas. Hum Gene Ther. 2009;20(2):125–36. doi:10.1089/hum.2008.116.
22. Salgaller ML, Bei R, Schlom J, Poole DJ, Robbins PF. Baculovirus recombinants expressing the human carcinoembryonic antigen gene. Cancer Res. 1993;53:2154–61.
23. Bei R, Guptill V, Masuelli L, Kashmiri SV, Muraro R, Frati L, Schlom J, Kantor J. The use of a cationic liposome formulation (DOTAP) mixed with a recombinant tumor-associated antigen to induce immune responses and protective immunity in mice. J Immunother. 1998;21(3):159–69. doi:10.1097/00002371-199805000-00001.
24. Woo SJ, Kim CH, Park MY, Kim HS, Sohn HJ, Park JS, Kim H-J, Oh S-T, Kim T-G. Co-administration of carcinoembryonic antigen and HIV TAT fusion protein with CpG-oligodeoxynucleotide induces potent antitumor immunity. Cancer Sci. 2008;99 (5):1034–9. doi:10.1111/j.1349-7006.2008.00760.x.
25. Dai S, Wan T, Wang B, Zhou X, Xiu F, Chen T, Wu Y, Cao X. More efficient induction of HLA-A*0201-restricted and carcinoembryonic antigen (CEA)–specific CTL response by immunization with exosomes prepared from heat-stressed CEA-positive tumor cells. Clin Cancer Res. 2005;11(20):7554–63. doi:10.1158/1078-0432.CCR-05-0810.
26. Vergati M, Intrivici C, Huen NY, Schlom J, Tsang KY. Strategies for cancer vaccine development. J Biomed Biotechnol. 2010;2010:1–13. doi:10.1155/2010/596432.
27. Huarte E, Sarobe P, Lu J, Casares N, Lasarte JJ, Dotor J, Ruiz M, Prieto J, Celis E, Borrás-Cuesta F. Enhancing immunogenicity of a CTL epitope from carcinoembryonic antigen by selective amino acid replacements. Clin Cancer Res. 2002;8:2336–44.
28. Tsang KY, Zhu M, Nieroda CA, Correale P, Zaremba S, Hamilton JM, Cole D, Lam C, Schlom J. Phenotypic stability of a cytotoxic T-cell line directed against an immunodominant epitope of human carcinoembryonic antigen. Clin Cancer Res. 1997;3:2439–49.
29. Zaremba S, Barzaga E, Zhu M, Soares N, Tsang KY, Schlom J. Identification of an enhancer agonist cytotoxic T lymphocyte peptide from human carcinoembryonic antigen. Cancer Res. 1997;57:4570–7.
30. Salazar E, Zaremba S, Arlen PM, Tsang KY, Schlom J. Agonist peptide from a cytotoxic T-lymphocyte epitope of human carcinoembryonic antigen stimulates production of tc1-type cytokines and increases tyrosine phosphorylation more efficiently than cognate peptide. Int J Cancer. 2000;85(6):829–38. doi:10.1002/(SICI)1097-0215(20000315)85:6<829:AID-IJC16>3.0.CO;2-K.
31. Palena C, Arlen P, Zeytin H, Greiner JW, Schlom J, Tsang KY. Enhanced expression of lymphotactin by CD8+ T cells is selectively induced by enhancer agonist peptides of tumor-associated antigens. Cytokine. 2003;24(4):128–42. doi:10.1016/j.cyto.2003.07.004.
32. Jia W, Zhang T, Huang H, Feng H, Wang S, Guo Z, Luo Z, Ji X, Cheng X, Zhao R, et al. Colorectal cancer vaccines: the current scenario and future prospects. Front Immunol. 2022;13:942235. doi:10.3389/fimmu.2022.942235.
33. Samanci A, Yi Q, Fagerberg J, Strigård K, Smith G, Rudén U, Wahren B, Mellstedt H. Pharmacological administration of granulocyte/macrophage-colony-stimulating factor is of significant importance for the induction of a strong humoral and cellular response in patients immunized with recombinant carcinoembryonic antigen. Cancer Immunol Immunother. 1998;47 (3):131–42. doi:10.1007/s002620050513.
34. Ullenhag GJ, Frödin JE, Jeddi-Tehrani M, Lidströmer N, Strigård K, Eriksson E, Choudhury A, Nilsson B, Rossmann ED, Mosolits S, et al. Durable carcinoembryonic antigen (CEA)-specific humoral and cellular immune responses in colorectal carcinoma patients vaccinated with recombinant CEA and granulocyte/macrophage colony-stimulating factor. Clin Cancer Res. 2004;10(10):3273–81. doi:10.1158/1078-0432.CCR-03-0706.
35. Staff C, Magnusson CG, Hojjat-Farsangi M, Mosolits S, Liljefors M, Frödin JE, Wahrén B, Mellstedt H, Ullenhag GJ. Induction of IgM, IgA and IgE antibodies in colorectal cancer patients vaccinated with a recombinant CEA protein. J Clin Immunol. 2012;32(4):855–65. doi:10.1007/s10875-012-9662-7.
36. Luo Y, O'Hagan D, Zhou H, Singh M, Ulmer J, Reisfeld RA, Primus, FJ, Xiang, R. Plasmid DNA encoding human carcinoembryonic antigen (CEA) adsorbed onto cationic microparticles

induces protective immunity against colon cancer in CEA-transgenic mice. Vaccine. 2003;21(17–18):1938–47. doi:10.1016/S0264-410X(02)00821-6.
37. Johansson S, Engström G, Winberg G, Hinkula J, Wahren B. Responses of mice immunized with a DNA vaccine encoding carcinoembryonic antigen (CEA). Vaccine. 2006;24(21):4572–5. doi:10.1016/j.vaccine.2005.08.044.
38. Johansson S, Ek M, Wahren B, Stout R, Liu M, Hallermalm K. Intracellular targeting of CEA results in Th1-type antibody responses following intradermal genetic vaccination by a needle-free jet injection device. ScientificWorldjournal. 2007;7:987–99. doi:10.1100/tsw.2007.138.
39. Li D, Hua S, Fan Y, Xu S, Duan X, Liu L, Che Y, Li S, Tan Y. DNA vaccine expressing repeated carcinoembryonic antigen (CEA)625–667 induces strong immunity in mice. Immunol Lett. 2011;135(1–2):124–8. doi:10.1016/j.imlet.2010.10.012.
40. Lima J, Jenkins C, Guerrero A, Triozzi PL, Shaw DR, Strong TV. A DNA vaccine encoding genetic fusions of carcinoembryonic antigen (CEA) and granulocyte/macrophage colony-stimulating factor (GM-CSF). Vaccine. 2005;23(10):1273–83. doi:10.1016/j.vaccine.2004.08.043.
41. Zhou ZF, Peretz Y, Chang Y, Miao DS, Li X, Prud'homme GJ. Intramuscular gene transfer of soluble B7.1/IgG(1) fusion cDNA induces potent antitumor immunity as an adjuvant for DNA vaccination. Cancer Gene Ther. 2003;10(6):491–9. doi:10.1038/sj.cgt.7700595.
42. Song K, Chang Y, Prud'homme GJ. IL-12 plasmid-enhanced DNA vaccination against carcinoembryonic antigen (CEA) studied in immune-gene knockout mice. Gene Ther. 2000;7(18):1527–35. doi:10.1038/sj.gt.3301274.
43. Bråve A, Hallengärd D, Gudmundsdotter L, Stout R, Walters R, Wahren B, Hallermalm K. Late administration of plasmid DNA by intradermal electroporation efficiently boosts DNA-primed T and B cell responses to carcinoembryonic antigen. Vaccine. 2009;27(28):3692–6. doi:10.1016/j.vaccine.2009.04.013.
44. Lopes A, Vandermeulen G, Préat V. Cancer DNA vaccines: current preclinical and clinical developments and future perspectives. J Exp Clin Canc Res. 2019;38(1):146. doi:10.1186/s13046-019-1154-7.
45. Kwilas AR, Donahue RN, Tsang KY, Hodge JW. Immune consequences of tyrosine kinase inhibitors that synergize with cancer immunotherapy. Cancer Cell Microenviron. 2015;2(1). doi:10.14800/ccm.677.
46. Xiang R, Primus FJ, Ruehlmann JM, Niethammer AG, Silletti S, Lode HN, Dolman CS, Gillies SD, Reisfeld RA. A dual-function DNA vaccine encoding carcinoembryonic antigen and CD40 ligand trimer induces T cell-mediated protective immunity against colon cancer in carcinoembryonic antigen-transgenic mice. J Immunol. 2001;167(8):4560–5. doi:10.4049/jimmunol.167.8.4560.
47. Niethammer AG, Primus FJ, Xiang R, Dolman CS, Ruehlmann JM, Ba Y, Gillies SD, Reisfeld RA. An oral DNA vaccine against human carcinoembryonic antigen (CEA) prevents growth and dissemination of Lewis lung carcinoma in CEA transgenic mice. Vaccine. 2001;20(3–4):421–9. doi:10.1016/S0264-410X(01)00362-0.
48. Gillies SD, Lan Y, Wesolowski JS, Qian X, Reisfeld RA, Holden S, Super M, Lo K-M. Antibody-IL-12 fusion proteins are effective in SCID mouse models of prostate and colon carcinoma metastases. J Immunol. 1998;160(12):6195–203. doi:10.4049/jimmunol.160.12.6195.
49. Xiang R, Silletti S, Lode HN, Dolman CS, Ruehlmann JM, Niethammer AG, Pertl U, Gillies SD, Primus FJ, Reisfeld RA, et al. Protective immunity against human carcinoembryonic antigen (CEA) induced by an oral DNA vaccine in CEA-transgenic mice. Clin Cancer Res. 2001;7(3 Suppl):856s–64.
50. Conry RM, Curiel DT, Strong TV, Moore SE, Allen KO, Barlow DL, Shaw DR, LoBuglio AF. Safety and immunogenicity of a DNA vaccine encoding carcinoembryonic antigen and hepatitis B surface antigen in colorectal carcinoma patients. Clin Cancer Res. 2002;8:2782–7.
51. Lutsiak ME, Semnani RT, De Pascalis R, Kashmiri SV, Schlom J, Sabzevari H. Inhibition of CD4(+)25+ T regulatory cell function implicated in enhanced immune response by low-dose cyclophosphamide. Blood. 2005;105(7):2862–8. doi:10.1182/blood-2004-06-2410.
52. Ruter J, Barnett BG, Kryczek I, Brumlik MJ, Daniel BJ, Coukos G, Zou, W, Curiel, TJ. Altering regulatory T cell function in cancer immunotherapy: a novel means to boost the efficacy of cancer vaccines. Front Biosci (Landmark Ed). 2009;14(5):1761–70. doi:10.2741/3338.
53. Staff C, Mozaffari F, Haller BK, Wahren B, Liljefors M. A Phase I safety study of plasmid DNA immunization targeting carcinoembryonic antigen in colorectal cancer patients. Vaccine. 2011;29(39):6817–22. doi:10.1016/j.vaccine.2010.12.063.
54. Rittig SM, Haentschel M, Weimer KJ, Heine A, Muller MR, Brugger W, Horger MS, Maksimovic O, Stenzl A, Hoerr I, et al. Intradermal vaccinations with RNA coding for TAA generate CD8 + and CD4+ immune responses and induce clinical benefit in vaccinated patients. Mol Ther. 2011;19(5):990–9. doi:10.1038/mt.2010.289.
55. McCann KJ, Mander A, Cazaly A, Chudley L, Stasakova J, Thirdborough S, King A, Lloyd-Evans P, Buxton E, Edwards C, et al. Targeting carcinoembryonic antigen with DNA vaccination: on-target adverse events link with immunologic and clinical outcomes. Clin Cancer Res. 2016;22(19):4827–36. doi:10.1158/1078-0432.CCR-15-2507.
56. Pierini S, Perales-Linares R, Uribe-Herranz M, Pol JG, Zitvogel L, Kroemer G, Facciabene A, Galluzzi L. Trial watch: DNA-based vaccines for oncological indications. Oncoimmunology. 2017;6(12):e1398878. doi:10.1080/2162402X.2017.1398878.
57. Kantor J, Irvine K, Abrams S, Kaufman H, DiPietro J, Schlom J. Antitumor activity and immune responses induced by a recombinant carcinoembryonic antigen-vaccinia virus vaccine. J Natl Cancer Inst. 1992;84(14):1084–91. doi:10.1093/jnci/84.14.1084.
58. Hodge JW, Schlom J. Comparative studies of a retrovirus versus a poxvirus vector in whole tumor-cell vaccines. Cancer Res. 1999;59:5106–11.
59. von Mehren M, Arlen P, Tsang KY, Rogatko A, Meropol N, Cooper HS, Davey M, McLaughlin S, Schlom J, Weiner LM. Pilot study of a dual gene recombinant avipox vaccine containing both carcinoembryonic antigen (CEA) and B7.1 transgenes in patients with recurrent CEA-expressing adenocarcinomas. Clin Cancer Res. 2000;6:2219–28.
60. Tsang KY, Zaremba S, Nieroda CA, Zhu MZ, Hamilton JM, Schlom J. Generation of human cytotoxic T cells specific for human carcinoembryonic antigen epitopes from patients immunized with recombinant vaccinia-CEA vaccine. J Natl Cancer Inst. 1995;87(13):982–90. doi:10.1093/jnci/87.13.982.
61. Hodge JW, McLaughlin JP, Kantor JA, Schlom J. Diversified prime and boost protocols using recombinant vaccinia virus and recombinant non-replicating avian pox virus to enhance T-cell immunity and antitumor responses. Vaccine. 1997;15(6–7):759–68. doi:10.1016/S0264-410X(96)00238-1.
62. Osada T, Yang XY, Hartman ZC, Glass O, Hodges BL, Niedzwiecki D, Morse MA, Lyerly HK, Amalfitano A, Clay TM, et al. Optimization of vaccine responses with an E1, E2b and E3-deleted Ad5 vector circumvents pre-existing anti-vector immunity. Cancer Gene Ther. 2009;16(9):673–82. doi:10.1038/cgt.2009.17.
63. Tang D-C, Shi Z, Curiel DT. Vaccination onto bare skin. Nature. 1997;388(6644):729–30. doi:10.1038/41917.
64. Bei R, Kantor J, Kashmiri SV, Abrams S, Schlom J. Enhanced immune responses and anti-tumor activity by baculovirus recombinant carcinoembryonic antigen (CEA) in mice primed with the recombinant vaccinia CEA. J Immunother Emphasis Tumor Immunol. 1994;16(4):275–82. doi:10.1097/00002371-199411000-00003.
65. Gabitzsch ES, Xu Y, Balint JP Jr., Hartman ZC, Lyerly HK, Jones FR. Anti-tumor immunotherapy despite immunity to adenovirus using a novel adenoviral vector Ad5 [E1-, E2b-]-CEA.

Cancer Immunol Immunother. 2010;59(7):1131–5. doi:10.1007/s00262-010-0847-8.
66. Morse MA, Chaudhry A, Gabitzsch ES, Hobeika AC, Osada T, Clay TM, Amalfitano A, Burnett BK, Devi GR, Hsu DS, et al. Novel adenoviral vector induces T-cell responses despite anti-adenoviral neutralizing antibodies in colorectal cancer patients. Cancer Immunol Immunother. 2013;62(8):1293–301. doi:10.1007/s00262-013-1400-3.
67. Hartman ZC, Wei J, Glass OK, Guo H, Lei G, Yang XY, Osada T, Hobeika A, Delcayre A, Le Pecq J-B, et al. Increasing vaccine potency through exosome antigen targeting. Vaccine. 2011;29(50):9361–7. doi:10.1016/j.vaccine.2011.09.133.
68. Facciabene A, Aurisicchio L, Elia L, Palombo F, Mennuni C, Ciliberto G, Monica NL. DNA and adenoviral vectors encoding carcinoembryonic antigen fused to immunoenhancing sequences augment antigen-specific immune response and confer tumor protection. Hum Gene Ther. 2006;17(1):81–92. doi:10.1089/hum.2006.17.81.
69. Facciabene A, Aurisicchio L, Elia L, Palombo F, Mennuni C, Ciliberto G, La Monica N. Vectors encoding carcinoembryonic antigen fused to the B subunit of heat-labile enterotoxin elicit antigen-specific immune responses and antitumor effects. Vaccine. 2007;26(1):47–58. doi:10.1016/j.vaccine.2007.10.060.
70. Sun Y, Wang S, Yang H, Wu J, Li S, Qiao G, Wang S, Wang X, Zhou X, Osada T, et al. Impact of synchronized anti-PD-1 with Ad-CEA vaccination on inhibition of colon cancer growth. Immunotherapy. 2019;11(11):953–66. doi:10.2217/imt-2019-0055.
71. Nayerossadat N, Maedeh T, Ali PA. Viral and nonviral delivery systems for gene delivery. Adv Biomed Res. 2012;1(1):27. doi:10.4103/2277-9175.98152.
72. Kotin RM, Linden RM, Berns KI. Characterization of a preferred site on human chromosome 19q for integration of adeno-associated virus DNA by non-homologous recombination. EMBO J. 1992;11(13):5071–8. doi:10.1002/j.1460-2075.1992.tb05614.x.
73. Hermonat PL. Adeno-associated virus inhibits human papillomavirus type 16: a viral interaction implicated in cervical cancer. Cancer Res. 1994;54:2278–81.
74. Triozzi PL, Aldrich W, Ponnazhagan S. Inhibition and promotion of tumor growth with adeno-associated virus carcinoembryonic antigen vaccine and Toll-like receptor agonists. Cancer Gene Ther. 2011;18(12):850–8. doi:10.1038/cgt.2011.54.
75. Di L, Zhu Y, Jia J, Yu J, Song G, Zhang J, Che L, Yang H, Han Y, Ma B, et al. Clinical safety of induced CTL infusion through recombinant adeno-associated virus-transfected dendritic cell vaccination in Chinese cancer patients. Clin Transl Oncol. 2012;14(9):675–81. doi:10.1007/s12094-012-0854-7.
76. Bhattacharya-Chatterjee M, Mukerjee S, Biddle W, Foon KA, Köhler H. Murine monoclonal anti-idiotype antibody as a potential network antigen for human carcinoembryonic antigen. J Immunol. 1990;145(8):2758–65. doi:10.4049/jimmunol.145.8.2758.
77. Pervin S, Chakraborty M, Bhattacharya-Chatterjee M, Zeytin H, Foon KA, Chatterjee SK. Induction of antitumor immunity by an anti-idiotype antibody mimicking carcinoembryonic antigen. Cancer Res. 1997;57:728–34.
78. Saha A, Baral RN, Chatterjee SK, Mohanty K, Pal S, Foon KA, Primus FJ, Krieg AM, Weiner GJ, Bhattacharya-Chatterjee M, et al. CpG oligonucleotides enhance the tumor antigen-specific immune response of an anti-idiotype antibody-based vaccine strategy in CEA transgenic mice. Cancer Immunol Immunother. 2006;55(5):515–27. doi:10.1007/s00262-005-0009-6.
79. Losman MJ, Novick KE, Goldenberg DM, Monestier M. Mimicry of a carcinoembryonic antigen epitope by a rat monoclonal anti-idiotype antibody. Int J Cancer. 1994;56(4):580–4. doi:10.1002/ijc.2910560419.
80. Schwegler C, Dorn-Beineke A, Nittka S, Stocking C, Neumaier M. Monoclonal anti-idiotype antibody 6G6.C4 fused to GM-CSF is capable of breaking tolerance to carcinoembryonic antigen (CEA) in CEA-transgenic mice. Cancer Res. 2005;65(5):1925–33. doi:10.1158/0008-5472.CAN-04-3591.
81. Pignatari GC, Takeshita D, Parise CB, Soares FA, de Moraes JZ, Han SW. Carcinoembryonic antigen (CEA) mimicry by an anti-idiotypic scFv isolated from anti-id 6.C4 hybridoma. J Biotechnol. 2007;127(4):615–25. doi:10.1016/j.jbiotec.2006.08.007.
82. Zanetti BF, Ferreira CP, de Vasconcelos JRC, Han SW. scFv6.C4 DNA vaccine with fragment C of tetanus toxin increases protective immunity against CEA-expressing tumor. Gene Ther. 2019;26(10):441–54. doi:10.1038/s41434-019-0062-y.
83. Zanetti BF, Ferreira CP, Vasconcelos JRC, Han SW. Adjuvant properties of IFN-γ and GM-CSF in the scFv6.C4 DNA vaccine against CEA-expressing tumors. Gene Ther. 2023;30(1–2):41–50. doi:10.1038/s41434-021-00270-w.
84. Denapoli PMA, Zanetti BF, dos Santos AA, de Moraes JZ, Han SW. Preventive DNA vaccination against CEA-expressing tumors with anti-idiotypic scFv6.C4 DNA in CEA-expressing transgenic mice. Cancer Immunol Immun. 2017;66(3):333–42. doi:10.1007/s00262-016-1940-4.
85. Morse MA, Crosby EJ, Force J, Osada T, Hobeika AC, Hartman ZC, Berglund P, Smith J, Lyerly HK. Clinical trials of self-replicating RNA-based cancer vaccines. Cancer Gene Ther. 2023;30(6):803–11. doi:10.1038/s41417-023-00587-1.
86. Morse MA, Hobeika AC, Osada T, Berglund P, Hubby B, Negri S, Niedzwiecki D, Devi GR, Burnett BK, Clay TM, et al. An alphavirus vector overcomes the presence of neutralizing antibodies and elevated numbers of Tregs to induce immune responses in humans with advanced cancer. J Clin Invest. 2010;120(9):3234–41. doi:10.1172/JCI42672.
87. Osada T, Berglund P, Morse MA, Hubby B, Lewis W, Niedzwiecki D, Yang XY, Hobeika A, Burnett B, Devi GR, et al. Co-delivery of antigen and IL-12 by Venezuelan equine encephalitis virus replicon particles enhances antigen-specific immune responses and antitumor effects. Cancer Immunol Immunother. 2012;61(11):1941–51. doi:10.1007/s00262-012-1248-y.
88. Myers AL, Krewet JA, Shah MR. Tumor immunity and prolonged survival following combined adenovirus-HSP72 and CEA-plasmid vaccination. Vaccine. 2005;23(27):3565–71. doi:10.1016/j.vaccine.2005.01.148.
89. Marshall JL, Hoyer RJ, Toomey MA, Faraguna K, Chang P, Richmond E, Pedicano JE, Gehan E, Peck RA, Arlen P, et al. Phase I study in advanced cancer patients of a diversified prime-and-boost vaccination protocol using recombinant vaccinia virus and recombinant nonreplicating avipox virus to elicit anti–carcinoembryonic antigen immune responses. J Clin Oncol. 2000;18(23):3964–73. doi:10.1200/JCO.2000.18.23.3964.
90. Marshall J, Tsang K, Arlen P, Ley L, Slack R, Toomey M, Chang P, Schlom J, editors. Phase I trial to determine the toxicity and immunologic efficacy of the addition of GM-CSF (GM) and IL-2 to the combination of vaccinia–CEA (V) and ALVAC–CEA (A) administered as a prime and boost in patients (PTS) with advanced CEA-bearing cancers. Proceedings of the 37th Annual Meeting of the American Society of Clinical Oncology; 2001; San Franciso, CA USA.
91. Schlom J, Sabzevari H, Grosenbach DW, Hodge JW. A triad of costimulatory molecules synergize to amplify T-cell activation in both vector-based and vector-infected dendritic cell vaccines. Artif Cells Blood Substit Immobil Biotechnol. 2003;31(2):193–228. doi:10.1081/BIO-120020178.
92. Aarts WM, Schlom J, Hodge JW. Vector-based vaccine/cytokine combination therapy to enhance induction of immune responses to a self-antigen and antitumor activity. Cancer Res. 2002;62:5770–7.
93. Marshall JL, Gulley JL, Arlen PM, Beetham PK, Tsang KY, Slack R, Hodge JW, Doren S, Grosenbach DW, Hwang J, et al. Phase I study of sequential vaccinations with fowlpox-CEA(6D)-TRICOM alone and sequentially with vaccinia-CEA(6D)-TRICOM, with and without granulocyte-macrophage colony-stimulating factor, in patients with carcinoembryonic antigen–

expressing carcinomas. J Clin Oncol. 2005;23(4):720–31. doi:10.1200/JCO.2005.10.206.
94. Duggan MC, Jochems C, Donahue RN, Richards J, Karpa V, Foust E, Paul B, Brooks T, Tridandapani S, Olencki T, et al. A phase I study of recombinant (r) vaccinia-CEA(6D)-TRICOM and rFowlpox-CEA(6D)-TRICOM vaccines with GM-CSF and IFN-α-2b in patients with CEA-expressing carcinomas. Cancer Immunol Immunother. 2016;65(11):1353–64. doi:10.1007/s00262-016-1893-7.
95. Kaufman HL, Kim-Schulze S, Manson K, DeRaffele G, Mitcham J, Seo KS, Kim DW, Marshall J. Poxvirus-based vaccine therapy for patients with advanced pancreatic cancer. J Transl Med. 2007;5(1):60. doi:10.1186/1479-5876-5-60.
96. Gatti-Mays ME, Redman JM, Donahue RN, Palena C, Madan RA, Karzai F, Bilusic M, Sater HA, Marté JL, Cordes LM, et al. A Phase I trial using a multitargeted recombinant adenovirus 5 (CEA/MUC1/Brachyury)-based immunotherapy vaccine regimen in patients with advanced cancer. Oncologist. 2020;25(6):479–e899. doi:10.1634/theoncologist.2019-0608.
97. Gatti-Mays ME, Strauss J, Donahue RN, Palena C, Del Rivero J, Redman JM, Madan RA, Marté JL, Cordes LM, Lamping E, et al. A phase I dose-escalation trial of BN-CV301, a recombinant poxviral vaccine targeting MUC1 and CEA with costimulatory molecules. Clin Cancer Res. 2019;25(16):4933–44. doi:10.1158/1078-0432.CCR-19-0183.
98. Redman J, Gandhy S, Gatti-Mays M, Sater HA, Tsai Y, Donahue R, Cordes L, Steinberg S, Marte J, McMahon S, et al. SO-28 a randomized phase II trial of mFOLFOX6-based standard of care alone or in combination with Ad-CEA vaccine plus avelumab in patients with previously untreated metastatic colorectal cancer. Ann Oncol. 2020;31:S227. doi:10.1016/j.annonc.2020.04.043.
99. Redman JM, Tsai YT, Weinberg BA, Donahue RN, Gandhy S, Gatti-Mays ME, Abdul Sater H, Bilusic M, Cordes L, Steinberg SM, et al. A randomized phase II trial of mFOLFOX6 + bevacizumab alone or with AdCEA vaccine + avelumab immunotherapy for untreated metastatic colorectal cancer. Oncologist. 2022;27(3):198–209. doi:10.1093/oncolo/oyab046.
100. Wu Y, Wan T, Zhou X, Wang B, Yang F, Li N, Chen G, Dai S, Liu S, Zhang M, et al. Hsp70-like protein 1 fusion protein enhances induction of carcinoembryonic antigen–specific CD8+ CTL response by dendritic cell vaccine. Cancer Res. 2005;65(11):4947–54. doi:10.1158/0008-5472.CAN-04-3912.
101. Morse MA, Deng Y, Coleman D, Hull S, Kitrell-Fisher E, Nair S, Schlom J, Ryback ME, Lyerly HK. A phase I study of active immunotherapy with carcinoembryonic antigen peptide (CAP-1)-pulsed, autologous human cultured dendritic cells in patients with metastatic malignancies expressing carcinoembryonic antigen. Clin Cancer Res. 1999;5:1331–8.
102. Liu KJ, Chao TY, Chang JY, Cheng AL, Ch'ang HJ, Kao WY, Wu Y-C, Yu W-L, Chung T-R, Whang-Peng J, et al. A phase I clinical study of immunotherapy for advanced colorectal cancers using carcinoembryonic antigen-pulsed dendritic cells mixed with tetanus toxoid and subsequent IL-2 treatment. J Biomed Sci. 2016;23(1):64. doi:10.1186/s12929-016-0279-7.
103. Buijs PR, Verhagen JH, van Eijck CH, van den Hoogen BG. Oncolytic viruses: from bench to bedside with a focus on safety. Hum Vaccin Immunother. 2015;11(7):1573–84. doi:10.1080/21645515.2015.1037058.
104. Kim SG, Park MY, Kim CH, Sohn HJ, Kim HS, Park JS, Kim H-J, Oh S-T, Kim T-G. Modification of CEA with both CRT and TAT PTD induces potent anti-tumor immune responses in RNA-pulsed DC vaccination. Vaccine. 2008;26(50):6433–40. doi:10.1016/j.vaccine.2008.08.072.
105. Morse MA, Nair SK, Mosca PJ, Hobeika AC, Clay TM, Deng Y, Boczkowski D, Proia A, Neidzwiecki D, Clavien# P-A, et al. Immunotherapy with autologous, human dendritic cells transfected with carcinoembryonic antigen mRNA. Cancer Invest. 2003;21(3):341–9. doi:10.1081/CNV-120018224.
106. Saha A, Chatterjee SK, Foon KA, Celis E, Bhattacharya-Chatterjee M. Therapy of established tumors in a novel murine model transgenic for human carcinoembryonic antigen and HLA-A2 with a combination of anti-idiotype vaccine and CTL peptides of carcinoembryonic antigen. Cancer Res. 2007;67(6):2881–92. doi:10.1158/0008-5472.CAN-06-3045.
107. Oh ST, Kim CH, Park MY, Won EH, Sohn HJ, Cho HI, Kang W-K, Hong Y-K, Kim T-G. Dendritic cells transduced with recombinant adenoviruses induce more efficient anti-tumor immunity than dendritic cells pulsed with peptide. Vaccine. 2006;24(15):2860–8. doi:10.1016/j.vaccine.2005.12.056.
108. Ojima T, Iwahashi M, Nakamura M, Matsuda K, Nakamori M, Ueda K, Naka T, Ishida K, Primus FJ, Yamaue H, et al. Successful cancer vaccine therapy for carcinoembryonic antigen (CEA)-expressing colon cancer using genetically modified dendritic cells that express CEA and T helper-type 1 cytokines in CEA transgenic mice. Int J Cancer. 2007;120(3):585–93. doi:10.1002/ijc.22298.
109. Morse MA, Hobeika AC, Osada T, Serra D, Niedzwiecki D, Lyerly HK, Clay TM. Depletion of human regulatory T cells specifically enhances antigen-specific immune responses to cancer vaccines. Blood. 2008;112(3):610–8. doi:10.1182/blood-2008-01-135319.
110. Kitadani J, Ojima T, Iwamoto H, Tabata H, Nakamori M, Nakamura M, Hayata K, Katsuda M, Miyajima M, Yamaue H, et al. Cancer vaccine therapy using carcinoembryonic antigen - expressing dendritic cells generated from induced pluripotent stem cells. Sci Rep. 2018;8(1):4569. doi:10.1038/s41598-018-23120-z.
111. Chavda VP, Yao Q, Vora LK, Apostolopoulos V, Patel CA, Bezbaruah R, Patel AB, Chen Z-S. Fast-track development of vaccines for SARS-CoV-2: the shots that saved the world. Front Immunol. 2022;13:961198. doi:10.3389/fimmu.2022.961198.
112. Bezbaruah R, Chavda VP, Nongrang L, Alom S, Deka K, Kalita T, Ali F, Bhattacharjee B, Vora L. Nanoparticle-based delivery systems for vaccines. Vaccines. 2022;10(11):1946. doi:10.3390/vaccines10111946.
113. Chavda VP, Apostolopoulos V. Mosaic receptor binding domain nanoparticles: towards fourth-generation vaccination. Nanomedicine. 2023;18(19):1223–6. doi:10.2217/nnm-2022-0316.
114. Sun D, Zhuang X, Xiang X, Liu Y, Zhang S, Liu C, Barnes S, Grizzle W, Miller D, Zhang H-G, et al. A novel nanoparticle drug delivery system: the anti-inflammatory activity of curcumin is enhanced when encapsulated in exosomes. Mol Ther. 2010;18(9):1606–14. doi:10.1038/mt.2010.105.
115. Dai S, Wei D, Wu Z, Zhou X, Wei X, Huang H, Li G. Phase I clinical trial of autologous ascites-derived exosomes combined with GM-CSF for colorectal cancer. Mol Ther. 2008;16(4):782–90. doi:10.1038/mt.2008.1.
116. Pocsfalvi G, Mammadova R, Ramos Juarez Ana P, Bokka R, Trepiccione F, Capasso G. COVID-19 and extracellular vesicles: an intriguing interplay. Kidney Blood Press Res. 2020;45(5):661–70. doi:10.1159/000511402.
117. Wang Z, Popowski KD, Zhu D, de Juan Abad BL, Wang X, Liu M, Lutz H, De Naeyer N, DeMarco CT, Denny TN, et al. Exosomes decorated with a recombinant SARS-CoV-2 receptor-binding domain as an inhalable COVID-19 vaccine. Nat Biomed Eng. 2022;6(7):791–805. doi:10.1038/s41551-022-00902-5.
118. Petrulio CA, Kaufman HL. Development of the PANVAC-VF vaccine for pancreatic cancer. Expert Rev Vaccines. 2006;5(1):9–19. doi:10.1586/14760584.5.1.9.
119. Skipper, M. Research highlights. Nat Rev Cancer. 2004;4(8):578. doi:10.1038/nrc1428.
120. Crosby EJ, Hobeika AC, Niedzwiecki D, Rushing C, Hsu D, Berglund P, Smith J, Osada T, Gwin III WR, Hartman ZC, et al. Long-term survival of patients with stage III colon cancer treated with VRP-CEA(6D), an alphavirus vector that increases the CD8+ effector memory T cell to Treg ratio. J Immunother Cancer. 2020;8(2):e001662. doi:10.1136/jitc-2020-001662.
121. Chakrabarti R, Chang Y, Song K, Prud'homme GJ. Plasmids encoding membrane-bound IL-4 or IL-12 strongly costimulate DNA vaccination against carcinoembryonic antigen (CEA). Vaccine. 2004;22(9–10):1199–205. doi:10.1016/j.vaccine.2003.09.023.

122. Bos R, van Duikeren S, van Hall T, Lauwen MM, Parrington M, Berinstein NL, McNeil B, Melief CJM, Verbeek JS, van der Burg SH, et al. Characterization of antigen-specific immune responses induced by canarypox virus vaccines. J Immunol. 2007;179(9):6115–22. doi:10.4049/jimmunol.179.9.6115.
123. Boehm AL, Higgins J, Franzusoff A, Schlom J, Hodge JW. Concurrent vaccination with two distinct vaccine platforms targeting the same antigen generates phenotypically and functionally distinct T-cell populations. Cancer Immunol Immunother. 2010;59(3):397–408. doi:10.1007/s00262-009-0759-7.
124. Jiang XP, Yang DC, Elliott RL, Head JF. Vaccination with a mixed vaccine of autogenous and allogeneic breast cancer cells and tumor associated antigens CA15-3, CEA and CA125–results in immune and clinical responses in breast cancer patients. Cancer Biother Radiopharm. 2000;15(5):495–505. doi:10.1089/cbr.2000.15.495.
125. Zhu MZ, Marshall J, Cole D, Schlom J, Tsang KY. Specific cytolytic T-cell responses to human CEA from patients immunized with recombinant avipox-CEA vaccine. Clin Cancer Res. 2000;6:24–33.
126. Hörig H, Lee DS, Conkright W, Divito J, Hasson H, LaMare M, Rivera A, Park D, Tine J, Guito K, et al. Phase I clinical trial of a recombinant canarypoxvirus (ALVAC) vaccine expressing human carcinoembryonic antigen and the B7.1 co-stimulatory molecule. Cancer Immunol Immunother. 2000;49(9):504–14. doi:10.1007/s002620000146.
127. Gulley JL, Madan RA, Tsang KY, Arlen PM, Camphausen K, Mohebtash M, Kamrava M, Schlom J, Citrin D. A pilot safety trial investigating a vector-based vaccine targeting carcinoembryonic antigen in combination with radiotherapy in patients with gastrointestinal malignancies metastatic to the liver. Expert Opin Biol Ther. 2011;11(11):1409–18. doi:10.1517/14712598.2011.615741.
128. Galanis E, Hartmann LC, Cliby WA, Long HJ, Peethambaram PP, Barrette BA, Kaur JS, Haluska PJ, Aderca I, Zollman PJ, et al. Phase I trial of intraperitoneal administration of an oncolytic measles virus strain engineered to express carcinoembryonic antigen for recurrent ovarian cancer. Cancer Res. 2010;70(3):875–82. doi:10.1158/0008-5472.CAN-09-2762.
129. Fong L, Hou Y, Rivas A, Benike C, Yuen A, Fisher GA, Davis MM, Engleman EG. Altered peptide ligand vaccination with Flt3 ligand expanded dendritic cells for tumor immunotherapy. Proc Natl Acad Sci USA. 2001;98(15):8809–14. doi:10.1073/pnas.141226398.