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COMMENTARY

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Immunotherapy regimens for metastatic colorectal carcinomas

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ABSTRACT

Metastatic colorectal cancer (mCRC) is a leading cause of cancer-related mortality with a 5-year overall survival rate of 13%. Despite recent advances in cancer immunotherapy, only the minority of CRC patients (<15%) with microsatellite instability can potentially benefit from immune checkpoint inhibitors, the only immunotherapy currently approved for mCRC. In that context, there is an unmet need to improve survival in mCRC. Our ever-increasing understanding of the immune system and its interactions with cancer has allowed development of multiple strategies to potentially improve outcomes in the majority of mCRC patients. Various approaches to manipulate patient immunity to recognize and kill colorectal cancer cells are being explored simultaneously, with combination therapies likely being the most effective. Ideally, therapies would target tumor-restricted antigens selectively found in tumors, but shielded from immune attack in normal tissues, to mount an effective cytotoxic T-cell response, while also overcoming cellular and molecular inhibitory pathways, self-tolerance, and T-cell exhaustion. Here, we provide a brief overview of the most promising immunotherapy candidates in mCRC and their strategies to produce a lasting immune response and clinical benefit in patients with mCRC.

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Colorectal cancer (CRC) is the 2nd leading cause of cancer related death in the United States.[1] At diagnosis, 20% of CRC patients have distant metastasis (mCRC) and half of all recurrences are in the form of metastatic disease.[1] The overall survival in mCRC is 13% at 5 years.[1] Excluding oligometastatic disease, the first-line treatment in mCRC consists of fluoropyrimidines and oxaliplatin or irinotecan chemotherapeutic agents.[2,3] Adding targeted agents like cetuximab, bevacizumab, or panitumumab offers a modest increase in overall survival.[4–7] The accelerated development of cancer immunotherapy over the last decade has revolutionized the current landscape for many cancer types. Here, we discuss some of the most promising developments in immune checkpoint inhibitor therapies and tumor vaccines for mCRC.

As with viral antigens, tumor-associated antigens (TAAs) are degraded into small peptides which are ultimately packaged in the groove of newly synthesized major histocompatibility complex (MHC) class I and II molecules and delivered as peptide-MHC complexes to the cell membrane of antigen presenting cells (APCs). T cell receptors (TCRs) on $CD8^+$ and $CD4^+$ T cells recognize these peptide-MHC complexes, and in the presence of the appropriate costimulatory signals from APCs, such as CD80, CD86, CD40, CD137, OX40L, and others, this in turn leads to activation of the T cells to proliferate, acquire effector functions such as cytokine production and cytolysis, and to produce long-lasting memory responses. In this context, development of cancer vaccines is often limited by the discovery of TAAs which are ubiquitously expressed by the cancer cells and absent from normal cells or immunologically compartmentalized to prevent damage to normal tissues from cytotoxic T cells (CTLs).

The efficacy of checkpoint inhibitors such as nivolumab and pembrolizumab has recently been established in microsatellite instability (MSI) CRC,[8,9] likely reflecting the immunological benefit derived from abundancy of mutation-associated neoantigens that serve as targets of effector T cells.[10-12] This hypothesis is further supported by the poor efficacy of checkpoint inhibitors in microsatellite stable (MSS) CRC. Moreover, distinct cancer immune phenotypes are increasingly being recognized, with tumors characterized by an "immune desert," lacking CTLs due to an absence of T-cell priming, tolerance, and/or immunologic ignorance due to a paucity of neoantigens or presentation by APCs, as the most difficult to treat.[13] Approximately 85% of CRC patients have MSS disease,[14] and often produce an abundance of transforming growth factor (TFG)-β contributing to immunologic tolerance by activation of $Foxp3^+$ regulatory T cells (Tregs)[15] as well as activation of stromal elements in the tumor microenvironment that inhibit CTL penetration, such as myeloid-derived suppressor cells (MDSCs).[16] Nevertheless, novel therapeutic combinations are being explored to increase the presentation of neoantigens in MSS CRC, such as dual immune checkpoint blockade with Durvalumab and Tremelimumab following targeted exposure to stereotactic body radiation therapy [ClinicalTrials.gov NCT03007407].[17]

Among the earliest modern attempts to harness the power of the immune system to fight cancer cells was the development of autologous cancer cell vaccines. These are comprised of

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autologous whole tumor cell lysates combined with immune adjuvants such as bacillus Calmette-Guérin (BCG), bacterial cell wall products, or virus-infected and irradiated tumor cells that are administered back to the patient to elicit adaptive antitumor immunity to multiple TAAs. OncoVAX (Vaccinogen, Inc.) utilizes irradiated, non-tumorigenic autologous tumor cells with BCG and had success in early phase clinical trials with improvement in disease-free and overall survival.[18] The pivotal Phase IIIb trial of OncoVAX [NCT02448173][19] started in 2015 under an FDA Special Protocol Assessment classification and is expected to complete enrollment by July, 2020. This approach may result in effective antitumor immunity but the personalized nature of this vaccine generation may pose a significant hurdle to its widespread adoption. Unfortunately, a similar approach using Newcastle disease virus-infected autologous tumors did not improve overall and disease-free survival in a randomized study.[20]

In addition to autologous tumor vaccines, immune responses can also be elicited with peptide, dendritic cell, DNA, or live attenuated viral vector-based immunotherapy. In the simplest form, tumor-associated peptides are isolated and administered to the patient with immunologic adjuvants. The peptide vaccine OCV-C02, containing epitopes derived from ring finger protein 43 (RNF43) and translocase of outer mitochondrial membrane 34 (TOMM34) was safe and tolerable, and produced CTL and delayed type hypersensitivity (DTH) responses in a Phase I study.[21] Hazama et al. tested the efficacy of a cocktail of five HLA-A*2402-restricted peptides combined with standard chemotherapy regimens such as FOLFOX or XELOX +/- bevacizumab in Japanese patients. This peptide cocktail included RNF43, TOMM34, KOC1, vascular endothelial growth factor receptor 1 (VEGFR1) and VEGFR2. The patients were categorized into HLA-A*2402 matched and unmatched groups. The expectation was that chemotherapy would produce an adjuvant effect by reducing the number of Tregs, but there was no significant difference in progression-free survival (PFS) and overall survival (OS) between the groups.[22] In the IMA910 study, peptides from 13 TAAs were injected into mCRC patients following 12 weeks of oxaliplatin-based therapy. The vaccine was administered following immunomodulation with low-dose cyclophosphamide (to deplete Tregs), either in combination with granulocyte monocyte colony stimulating factor (GM-CSF) or with GM-CSF and topically-applied imiquimod (a toll-like receptor [TLR] 7 agonist). Responders developed CTL responses against multiple peptides and had better disease control rate (18% vs. 2% at 6 months; $p = 0.012$) and PFS (HR 0.652; $p = 0.039$).[23] The efficacy of this approach in a large, randomized clinical trial with prospectively defined endpoints has not yet been completed.

Dendritic cells (DC) play an important role in antigen presentation and activation of CTLs. The development of an immunologic response with peptide vaccines is dependent upon uptake and presentation by DCs or other APCs. Others have employed an alternative approach in which a patient's DCs are collected and pulsed with antigens *ex vivo*, and following maturation, engineered DCs are administered to the patient as a cancer vaccine to elicit immune responses against tumors. This approach is similar to the only FDA-approved cancer vaccine, Sipuleucel-T for castration-resistant prostate cancer.[24] Carcinoembryonic antigen (CEA), a common CRC tumor marker, has been used with a DC-based vaccine approach to elicit antitumor immune responses, however, in a Phase II trial of the vaccine, the PFS and OS were not superior to best supportive care in mCRC.[25] On the other hand, DNA-based vaccines can be delivered to APCs as naked DNA plasmids, often combined with immunologic adjuvants such as IL-12, IL-15, and/or GM-CSF. Upon delivery to mammalian cells, DNA plasmids induce expression of specific antigens that are designed to activate the immune system directly by delivery into DCs or indirectly into parenchymal cells leading to antigen expression and subsequent uptake by APCs. CEA, nuclear oncoprotein MYB, heat shock protein 105, guanylyl cyclase C (GUCY2C), and human telomerase reverse transcriptase (hTERT) -based DNA vaccines have successfully induced antitumor immunity in preclinical models of CRC, alone or in combination with other vaccines.[26–30] CEA alone in its glycosylated and secreted form (*tetwt*CEA) or non-glycosylated form (CEA66), as well as in combination with immunogenic foreign antigens, has been tested in Phase I clinical trials with acceptable safety profiles.[31-33] hTERT elongates telomeric DNA ends and its expression is upregulated in 85–90% of human cancers, whereas it is absent in most normal somatic cells.[34,35] A Phase I trial [NCT02960594] is underway using either hTERT DNA vaccine alone or in combination with IL-12 DNA to stimulate immune responses. The clinical efficacy of a DNA vaccine using the same DNA vaccine platform to target human papillomavirus (HPV) antigens in cervical intraepithelial neoplasia (CIN),[36] suggests that the hTERT DNA vaccine may also prove to be effective in hTERT-expressing malignancies. DNA vaccines are generally safe, tolerable, easy to manufacture, and able to induce both humoral and cytotoxic immunity but their intracellular delivery requires electroporation to temporarily increase the permeability of the cell membranes.[37] Moreover, the immunogenicity and efficacy of DNA-based vaccines used as single agents appears to be low, while combinations with other vaccines may be significantly more effective than either single agent alone.[30]

It has long been recognized that vaccine delivery using live attenuated viral or bacterial vectors is likely the most robust way to induce immune response to TAAs and produce effective antitumor immunity. The potential drawbacks include the presence of host immunity against the vector, cost of production, and a potential for pathogenesis as well as insertional mutagenesis. Among viral vectors, adenovirus and poxviruses (vaccinia, fowlpox, canarypox, etc) are the most commonly explored, while *Listeria monocytogenes* has been examined as a bacterial vector for cancer vaccines. CEA is by far the most commonly targeted antigen in mCRC and is under development with several different vectors. In a Phase I study, CEA was used in a prime-boost approach with replication defective fowlpox and vaccinia vectors, all of which also expressed the genes for three T-cell costimulatory molecules (B7.1, ICAM-1, LFA-3 collectively called TRICOM).[38] The study tested fowlpox-CEA-TRICOM and vaccinia-CEA-TRICOM alone, together, or in combination with GM-CSF in 58 CEA-expressing cancer patients.[38] The vaccines were safe, tolerable, and generated T-cell responses to CEA in most patients. Importantly, 40% of patients had stable disease at 4 months and one patient achieved pathological complete

response.[38] A Phase II study evaluated safety, tolerability, CEA-specific immunity, and objective clinical responses following administration of a non-replicating canarypox virus expressing CEA and B7.1 (ALVAC-CEA/B7.1) administered concurrently or sequentially with systemic chemotherapy (IFL/FOLIRI) and/or tetanus toxoid (TT) in 118 patients with mCRC. Gastrointestinal and hematologic serious adverse events (SAEs) were seen in 30 and 24 patients, respectively. The majority of patients across all groups developed a CEA-specific T-cell response which was not attenuated by chemotherapy. The total objective response was observed in 44.7% of subjects in the chemotherapy + ALVAC group (n = 38), 31.3% of subjects in the ALVAC + TT + chemotherapy group (n = 32), and 44.1% of subjects in the ALVAC + chemotherapy group (n = 34).[39] Overall, the study demonstrated the feasibility of combination chemoimmunotherapy and provides rationale to develop combinations intended to achieve clinical remission in mCRC.[39] Another study is currently evaluating a combination of adenovirus-CEA vaccine with avelumab (a checkpoint inhibitor) with or without chemotherapy in previously untreated mCRC (NCT03050814).[40]

Beyond CEA, mucin (MUC1), epithelial cell adhesion molecule (EpCAM), the oncofetal antigen 5T4, and guanylyl cyclase C (GUCY2C) have also been in clinical development. MUC1 is normally expressed on the lining of human colon and is expressed in a modified form on advanced polyps and CRC. MUC1 with poly-ICLC adjuvant was tested in a Phase I/II setting in patients with a history of adenomatous polyps and found to be highly immunogenic in 43.6% of patients, whereas a high frequency of pre-vaccination MDSCs were found to be associated with immune non-responders.[41] EpCAM is highly expressed in many epithelial cancers including CRC.[42] EpCAM protein produced in a baculovirus expression system and conjugated to alum, was administered to 7 CRC patients with GM-CSF, inducing a Th1-biased humoral and cellular immune response.[43] Future studies are needed to demonstrate objective clinical responses in patients. 5T4 is a trophoblast glycoprotein with high-level expression in human adenocarcinomas, including CRC where it is found in more than 90% of tumors.[44] A poxvirus-based 5T4 vaccine (TroVax) was recently tested in mCRC patients with stable disease at completion of standard chemotherapy.[45] Of the 52 patients in the study, 9 were randomized to surveillance alone, 9 to cyclophosphamide alone, 19 to TroVax only, and 18 to a combination of TroVax and cyclophosphamide. TroVax was safe, well tolerated, and resulted in significantly improved PFS (5.6 vs 2.4 months) and OS (20 vs 10.3 months). Interestingly, the combination of TroVax and cyclophosphamide was not superior to TroVax alone. These data look promising but a larger sample size is required to demonstrate efficacy of TroVax without the need for cyclophosphamide.

GUCY2C, a cyclic GMP (cGMP) synthesizing protein is universally expressed in apical brush border membranes of intestinal cells and GUCY2C protein is found in nearly all primary and metastatic CRCs, with uniform expression by tumor cells, regardless of location or grade.[46–48] An adenovirus vector (Ad5)-based vaccine expressing GUCY2C conjugated to the **Pan DR e**pitope PADRE (Ad5-GUCY2C-PADRE) was evaluated in humans in an open-label, single-dose feasibility study in early-stage colorectal cancer patients [NCT01972737].[49,50] The vaccine was safe and immunogenic, producing GUCY2C-specific $CD8^+$ CTL responses in 40% of patients. A larger Phase II study is planned to begin in 2018 to explore the vaccine's efficacy for GUCY2C-expressing gastrointestinal malignancies.

Interest in cancer immunotherapy development began in 1893 with William Coley,[51] but little progress was made over the following century. Now, our understanding of the molecular and cellular mechanisms and complexities of the immune system has advanced significantly and the prospects of successful cancer immunotherapy development grow brighter with the pace of scientific discovery. The effectiveness of checkpoint inhibition in MSI tumors, including CRC, provides evidence that characterizing molecular and immunological subtypes may be important in determining patients most capable of inducing effective tumor immunity or selecting the immunotherapeutic approach most favorable for a given patient. Unfortunately, more than 95% of mCRC patients have MSS disease and cannot be treated with current immunotherapy options.[52] A growing body of evidence suggests that effective antitumor immunity in mCRC may be achieved using experimental cancer vaccines in combination regimens that promote depletion of Tregs and MDSCs, and block checkpoints, that prevent the induction or intratumoral activity of T-cell responses.

Disclosure of potential conflicts of interest

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References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. CA Cancer J Clin. 2017;67:7–30. doi:10.3322/caac.21387. PMID:28055103.
2. Fuchs CS, Marshall J, Mitchell E, Wierzbicki R, Ganju V, Jeffery M, Schulz J, Richards D, Soufi-Mahjoubi R, Wang B, et al. Randomized, controlled trial of irinotecan plus infusional, bolus, or oral fluoropyrimidines in first-line treatment of metastatic colorectal cancer: Results from the BICC-C study. J Clin Oncol. 2007;25:4779–86. doi:10.1200/JCO.2007.11.3357. PMID:17947725.
3. Cassidy J, Tabernero J, Twelves C, Brunet R, Butts C, Conroy T, Debraud F, Figer A, Grossmann J, Sawada N, et al. XELOX (Capecitabine Plus Oxaliplatin): Active first-line therapy for patients with metastatic colorectal cancer. J Clin Oncol. 2004;22:2084–91. doi:10.1200/JCO.2004.11.069. PMID:15169795.
4. Kabbinavar F, Hurwitz HI, Fehrenbacher L, Meropol NJ, Novotny WF, Lieberman G, Griffing S, Bergsland E. Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/Leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. J Clin Oncol. 2003;21:60–5. doi:10.1200/JCO.2003.10.066. PMID:12506171.
5. Cutsem EV, Nowacki M, Lang I, Cascinu S, Shchepotin I, Maurel J, Rougier P, Cunningham D, Nippgen J, Köhne C. Randomized phase

III study of irinotecan and 5-FU/FA with or without cetuximab in the first-line treatment of patients with metastatic colorectal cancer (mCRC): The CRYSTAL trial. J Clin Oncol. 2007;25:4000.
6. Bokemeyer C, Bondarenko I, Makhson A, Hartmann JT, Aparicio J, Braud F, Donea S, Ludwig H, Schuch G, Stroh C, et al. Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer. J Clin Oncol. 2009;27:663–71. doi:10.1200/JCO.2008.20.8397. PMID:19114683.
7. Douillard J-Y, Siena S, Cassidy J, Tabernero J, Burkes R, Barugel M, Humblet Y, Bodoky G, Cunningham D, Jassem J, et al. Randomized, Phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: The PRIME study. J Clin Oncol. 2010;28:4697–705. doi:10.1200/JCO.2009.27.4860. PMID:20921465.
8. Overman MJ, McDermott R, Leach JL, Lonardi S, Lenz HJ, Morse MA, Desai J, Hill A, Axelson M, Moss RA, et al. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. Lancet Oncol. 2017;18(9):1182–91. doi:10.1016/S1470-2045(17)30422-9. PMID:28734759.
9. Le DT, Durham JN, Smith KN, Wang H, Bartlett BR, Aulakh LK, Lu S, Kemberling H, Wilt C, Luber BS, et al. Mismatch-repair deficiency predicts response of solid tumors to PD-1 blockade. Science. 2017;357 (6349):409–13. doi:10.1126/science.aan6733. PMID:28596308.
10. Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, Walsh LA, Postow MA, Wong P, Ho TS, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. N Engl J Med. 2014;371:2189–99. doi:10.1056/NEJMoa1406498. PMID:25409260.
11. Andre T, Lonardi S, Wong KYM, Morse M, McDermott RS, Hill AG, et al. Combination of nivolumab (nivo) + ipilimumab (ipi) in the treatment of patients (pts) with deficient DNA mismatch repair (dMMR)/high microsatellite instability (MSI-H) metastatic colorectal cancer (mCRC): CheckMate 142 study. J Clin Oncol. 2017;35:3531.
12. Maby P, Tougeron D, Hamieh M, Mlecnik B, Kora H, Bindea G, Angell HK, Fredriksen T, Elie N, Fauquembergue E, et al. Correlation between density of CD8+ T-cell infiltrate in microsatellite unstable colorectal cancers and frameshift mutations: A rationale for personalized immunotherapy. Cancer Res. 2015;75:3446–55. doi:10.1158/0008-5472.CAN-14-3051. PMID:26060019.
13. Chen DS, Mellman I. Elements of cancer immunity and the cancer-immune set point. Nature. 2017;541:321–30. doi:10.1038/nature21349. PMID:28102259.
14. Vilar E, Gruber SB. Microsatellite instability in colorectal cancer [mdash]the stable evidence. Nat Rev Clin Oncol. 2010;7:153–62. doi:10.1038/nrclinonc.2009.237. PMID:20142816.
15. Coombes JL, Siddiqui KRR, Arancibia-Cárcamo CV, Hall J, Sun CM, Belkaid Y, Powrie F. A functionally specialized population of mucosal CD103$^+$DCs induces Foxp3$^+$regulatory T cells via a TGF-β– and retinoic acid–dependent mechanism. J Exp Med. 2007;204:1757–64. doi:10.1084/jem.20070590. PMID:17620361.
16. Feig C, Jones JO, Kraman M, Wells RJB, Deonarine A, Chan DS, Connell CM, Roberts EW, Zhao Q, Caballero OL, et al. Targeting CXCL12 from FAP-expressing carcinoma-associated fibroblasts synergizes with anti–PD-L1 immunotherapy in pancreatic cancer. Proc Natl Acad Sci. 2013;110:20212–7. doi:10.1073/pnas.1320318110. PMID:24277834.
17. NSABP Foundation Inc. Study of durvalumab and tremelimumab after radiation for microsatellite stable metastatic colorectal cancer progressing on chemotherapy In: ClinicalTrialsgov [Internet] Bethesda (MD): National library of medicine (US) 2000- [cited 2017 September 19] Available from: https://clinicaltrialsgov/ct2/show/NCT03007407NLMIdentifier:NCT03007407.
18. Hanna MG, Hoover HC, Vermorken JB, Harris JE, Pinedo HM. Adjuvant active specific immunotherapy of stage II and stage III colon cancer with an autologous tumor cell vaccine: first randomized phase III trials show promise. Vaccine. 2001;19:2576–82. doi:10.1016/S0264-410X(00)00485-0. PMID:11257395.
19. Vaccinogen Inc. A multicenter study of active specific immunotherapy with OncoVax® in patients with stage II colon cancer In: ClinicalTrialsgov [Internet] Bethesda (MD): National Library of Medicine (US) 2000- [cited 2017 September 19] Available from: https://clinicaltrialsgov/ct2/show/NCT02448173NLMIdentifier:NCT02448173.
20. Schulze T, Kemmner W, Weitz J, Wernecke KD, Schirrmacher V, Schlag PM. Efficiency of adjuvant active specific immunization with Newcastle disease virus modified tumor cells in colorectal cancer patients following resection of liver metastases: results of a prospective randomized trial. Cancer Immunol Immunother. 2008;58:61. doi:10.1007/s00262-008-0526-1. PMID:18488223.
21. Taniguchi H, Iwasa S, Yamazaki K, Yoshino T, Kiryu C, Naka Y, Liew EL, Sakata Y. Phase 1 study of OCV-C02, a peptide vaccine consisting of two peptide epitopes for refractory metastatic colorectal cancer. Cancer Sci. 2017;108:1013–21. doi:10.1111/cas.13227. PMID:28266765.
22. Hazama S, Nakamura Y, Tanaka H, Hirakawa K, Tahara K, Shimizu R, Ozasa H, Etoh R, Sugiura F, Okuno K, et al. A phase II study of five peptides combination with oxaliplatin-based chemotherapy as a first-line therapy for advanced colorectal cancer (FXV study). J Transl Med. 2014;12:108. doi:10.1186/1479-5876-12-108. PMID:24884643.
23. Mayer F, Mayer-Mokler A, Nowara E, Torday L, Ludwig J, Kuttruff S, Weinschenk T, Walter S, Singh H, Reinhardt C. A phase I/II trial of the multipeptide cancer vaccine IMA910 in patients with advanced colorectal cancer (CRC). J Clin Oncol. 2012;30:555. doi:10.1200/jco.2012.30.4_suppl.555.
24. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, Redfern CH, Ferrari AC, Dreicer R, Sims RB, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med. 2010;363:411–22. doi:10.1056/NEJMoa1001294. PMID:20818862.
25. Maurel J, Caballero-Baños M, Mila J, Tabera J, Varea S, Vilana R, Ayuso JR, Pagés M, Carrera G, Cuatrecasas M, et al. Phase II randomized trial of autologous tumor lysate dendritic cell vaccine (ADC) plus best supportive care (BSC) compared with BSC, in pre-treated advanced colorectal cancer patients. J Clin Oncol. 2015;33:3048.
26. Cross RS, Malaterre J, Davenport AJ, Carpinteri S, Anderson RL, Darcy PK, Ramsay RG. Therapeutic DNA vaccination against colorectal cancer by targeting the MYB oncoprotein. Clin Trans Immunol. 2015;4:e30. doi:10.1038/cti.2014.29.
27. Miyazaki M, Nakatsura T, Yokomine K, Senju S, Monji M, Hosaka S, Komori H, Yoshitake Y, Motomura Y, Minohara M, et al. DNA vaccination of HSP105 leads to tumor rejection of colorectal cancer and melanoma in mice through activation of both CD4+ T cells and CD8+ T cells. Cancer Sci. 2005;96:695–705. doi:10.1111/j.1349-7006.2005.00093.x. PMID:16232202.
28. Yan J, Pankhong P, Shin TH, Obeng-Adjei N, Morrow MP, Walters JN, Khan AS, Sardesai NY, Weiner DB. Highly optimized DNA vaccine targeting human telomerase reverse transcriptase stimulates potent antitumor immunity. Cancer Immunol Res. 2013;1:179–89. doi:10.1158/2326-6066.CIR-13-0001. PMID:24777680.
29. Turriziani M, Fantini M, Benvenuto M, Izzi V, Masuelli L, Sacchetti P, Modesti A, Bei R. Carcinoembryonic antigen (CEA)-based cancer vaccines: Recent patents and antitumor effects from experimental models to clinical trials. Recent Pat Anticancer Drug Discov. 2012;7:265–96. doi:10.2174/157489212801820020. PMID:22630596.
30. Xiang B, Baybutt TR, Berman-Booty L, Magee MS, Waldman SA, Alexeev VY, Snook AE. Prime-boost immunization eliminates metastatic colorectal cancer by producing high-avidity effector CD8$^+$ T cells. J Immunol. 2017;198:3507–14. PMID:28341670.
31. McCann KJ, Mander A, Cazaly A, Chudley L, Stasakova J, Thirdborough SM, 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;22:4827–36. doi:10.1158/1078-0432.CCR-15-2507. PMID:27091407.
32. 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;29:6817–22. doi:10.1016/j.vaccine.2010.12.063. PMID:21195077.
33. 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. PMID:12231517.
34. Shay JW, Bacchetti S. A survey of telomerase activity in human cancer. Eur J Cancer. 1997;33:787–91. doi:10.1016/S0959-8049(97)00062-2. PMID:9282118.
35. Kim N, Piatyszek M, Prowse K, Harley C, West M, Ho P, Coviello GM, Wright WE, Weinrich SL, Shay JW. Specific association of human telomerase activity with immortal cells and cancer. Science. 1994;266:2011–5. doi:10.1126/science.7605428. PMID:7605428.
36. Trimble CL, Morrow MP, Kraynyak KA, Shen X, Dallas M, Yan J, Edwards L, Parker RL, Denny L, Giffear M, et al. Safety, efficacy, and immunogenicity of VGX-3100, a therapeutic synthetic DNA vaccine targeting human papillomavirus 16 and 18 E6 and E7 proteins for cervical intraepithelial neoplasia 2/3: a randomised, double-blind, placebo-controlled phase 2b trial. Lancet. 2015;386:2078–88. doi:10.1016/S0140-6736(15)00239-1. PMID:26386540.
37. Aihara H, Miyazaki JI. Gene transfer into muscle by electroporation in vivo. Nat Biotech. 1998;16:867–70. doi:10.1038/nbt0998-867.
38. 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;23:720–31. doi:10.1200/JCO.2005.10.206. PMID:15613691.
39. Kaufman HL, Lenz HJ, Marshall J, Singh D, Garett C, Cripps C, Moore M, Von Mehren M, Dalfen R, Heim WJ, et al. Combination chemotherapy and ALVAC-CEA/B7.1 vaccine in patients with metastatic colorectal cancer. Clin Cancer Res. 2008;14:4843–9. doi:10.1158/1078-0432.CCR-08-0276. PMID:18676757.
40. National Cancer Institute (NCI). Standard of care alone or in combination with Ad-CEA vaccine and avelumab in people with previously untreated metastatic colorectal cancer QUILT-2004 In: ClinicalTrials-gov [Internet] Bethesda (MD): National library of medicine (US) 2000- [cited 2017 September 19] Available from: https://clinicaltrials gov/ct2/show/NCT03050814NLMIdentifier:NCT03050814.
41. Kimura T, McKolanis JR, Dzubinski LA, Islam K, Potter DM, Salazar AM, Schoen RE, Finn OJ. MUC1 vaccine for individuals with advanced adenoma of the colon: A cancer immunoprevention feasibility study. Cancer Prev Res. 2013;6:18–26. doi:10.1158/1940-6207.CAPR-12-0275.
42. Balzar M, Winter MJ, de Boer CJ, Litvinov SV. The biology of the 17-1A antigen (Ep-CAM). J Mol Med (Berl). 1999;77:699–712. doi:10.1007/s001099900038. PMID:10606205.
43. Mosolits S, Markovic K, Frödin JE, Virving L, Magnusson CGM, Steinitz M, Fagerberg J, Mellstedt H. Vaccination with Ep-CAM protein or Anti-idiotypic antibody induces Th1-biased response against MHC class I- and II-Restricted Ep-CAM epitopes in colorectal carcinoma patients. Clin Cancer Res. 2004;10:5391–402. doi:10.1158/1078-0432.CCR-04-0425. PMID:15328177.
44. Starzynska T, Marsh PJ, Schofield PF, Roberts SA, Myers KA, Stern PL. Prognostic significance of 5T4 oncofetal antigen expression in colorectal carcinoma. Br J Cancer. 1994;69:899–902. doi:10.1038/bjc.1994.173. PMID:8180020.
45. Scurr M, Pembroke T, Bloom A, Roberts D, Thomson A, Smart K, Bridgeman H, Adams R, Brewster A, Jones R, et al. Effect of modified vaccinia ankara-5T4 and low-dose cyclophosphamide on antitumor immunity in metastatic colorectal cancer: A randomized clinical trial. JAMA Oncol. 2017;3:e172579. doi:10.1001/jamaoncol.2017.2579. PMID:28880972.
46. Almenoff JS, Williams SI, Scheving LA, Judd AK, Schoolnik GK. Ligand-based histochemical localization and capture of cells expressing heat-stable enterotoxin receptors. Mol Microbiol. 1993;8:865–73. doi:10.1111/j.1365-2958.1993.tb01633.x. PMID:8102772.
47. Carrithers SL, Parkinson SJ, Goldstein S, Park P, Robertson DC, Waldman SA. Escherichia coli heat-stable toxin receptors in human colonic tumors. Gastroenterology. 1994;107:1653–61. doi:10.1016/0016-5085(94)90804-4. PMID:7958675.
48. Carrithers SL, Barber MT, Biswas S, Parkinson SJ, Park PK, Goldstein SD, Waldman SA. Guanylyl cyclase C is a selective marker for metastatic colorectal tumors in human extraintestinal tissues. Proc Natl Acad Sci U S A. 1996;93:14827–32. doi:10.1073/pnas.93.25.14827. PMID:8962140.
49. Snook A, Baybutt T, Mastrangelo M, Lewis N, Goldstein S, Kraft W, Oppong Y, Hyslop T, Myers R, Alexeev V. A Phase I study of AD5-GUCY2C-PADRE in stage I and II colon cancer patients. J Immunother Cancer. 2015;3:P450. doi:10.1186/2051-1426-3-S2-P450.
50. Robinson E, Bartal A, Mekori T. Results of postoperative treatment of colorectal cancer by radiotherapy, chemotherapy and immunotherapy. Recent Results Cancer Res. 1980;75:80–7. doi:10.1007/978-3-642-81491-4_13. PMID:7232842.
51. Coley WB. The treatment of malignant tumors by repeated inoculations of erysipelas: with a report of ten original cases. Am J Med Sci. 1893;105:487–511. doi:10.1097/00000441-189305000-00001.
52. Koopman M, Kortman GAM, Mekenkamp L, Ligtenberg MJL, Hoogerbrugge N, Antonini NF, Punt CJA, van Krieken JHJM. Deficient mismatch repair system in patients with sporadic advanced colorectal cancer. Br J Cancer. 2009;100:266–73. doi:10.1038/sj.bjc.6604867. PMID:19165197.