**in vivo** *24*: 607-628 (2010)

*Review*

# Colon Cancer Vaccines: An Update

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**Abstract.** *Despite advances in research and treatment modalities, colorectal cancer still accounts for around half a million deaths yearly worldwide. Traditional and even newer pharmaceutical therapeutic regimens are limited in terms of tolerance, efficacy and cross-resistance. Additional non-cross resistant therapies with non-overlapping toxicities are needed to improve the outcome for patients with colorectal cancer. Cancer vaccines, designed to activate immune effectors (T-cells and antibodies) to prevent recurrence or treat advanced cancers, have now demonstrated clinical benefit in prostate cancer and lymphoma. Because immune effector infiltration into colon tumours is associated with improved clinical outcome, vaccines intended to activate immune responses against colon cancer have generated significant interest. This review discusses data supportive of the immune responsiveness of colorectal cancer, as well as the current status of colon cancer vaccines under development including those based on whole tumour cells or lysates, peptide or protein antigens, anti-idiotype antibodies, viral vectors, and dendritic cells. We also discuss challenges to colon cancer vaccine development, such as tumour associated mechanisms for immune evasion, and how future strategies may address these challenges.*

Colorectal cancer is the third most common cancer diagnosis and cause of cancer-related death in the US and accounts for more than 1 million cases diagnosed each year worldwide. It represents more than half of all intestinal malignancies (52.6%) and has a poor 5-year survival (64% for cancer of all stages at diagnosis but only 11% for metastatic cancer) with 50-60% metastatic rate, despite advances in treatment modalities (1). Surgery is the only potentially curative treatment choice available, but is rarely sufficient in patients with regionally advanced or metastatic disease. Traditional pharmaceutical therapeutic regimens including chemo-radiotherapy are limited in terms of toxicity and lack of tumour specificity and at best reduce the death rate for stage III disease by 30-40% (2) and prolong survival modestly in metastatic disease. Alternative therapeutic strategies are clearly needed and data supportive of the concept that colorectal tumours are immunoresponsive has led to the application of immunotherapy in the management of colorectal cancer.

*Conflict of interest:* The Authors have no potential conflicts of interest.

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*Key Words:* Colon cancer immunogenicity, dendritic cell vaccine, autologous vaccine, anti-idiotype vaccine, review.

## Colorectal Cancer Immunology and Immune Evasion

Before discussing the technologies developed to activate immune responses against colon cancer, it is important to describe the current understanding of colorectal cancer immunology and immune evasion.

*Colorectal cancer activates immune responses.* Colorectal tumours clearly harbour immunogenic proteins. At least ten tumour-associated antigens and thirty-five major histocompatibility complex (MHC) restricted epitopes derived from tumour antigens have been identified, potential targets for T-cell mediated adaptive immune response (3, 4). Antigenic stimulation leads to the generation of a small population of antigen-specific memory T-cells, which remain in the tissue (5). Greater numbers of infiltrating memory T-cells have been linked to attenuation of metastatic potential (6). Intra-tumour lymphocytic infiltration has been shown not only to inhibit tumour growth (7) but also to improve

survival (8-10), which suggests that the immune system is capable of mounting an immune response to colorectal cancer but is not always effective in sustaining it or preventing tumour progression (11). Importantly, the patients with the best survival had tumours with greater numbers of infiltrating CD8 and granzyme B-expressing T-cells.

*How colorectal cancer inhibits immune responses*. Similar to other types of cancer, colorectal cancer arises through evasion of the host's immuno-surveillance, as a result of weak immunogenicity or immunosuppressive effects of tumour cells (12-15). Restoration of antitumour immunological function after tumour resection has been observed in several studies (16, 17) suggesting that colorectal cancer has a direct immunosuppressive effect at a molecular and cellular level with suppression of cell mediated immunity [Th1 $CD4^+$ T lymphocytes producing cytokines, interleukin 2 (IL2), interferon (IFN) gamma and tumour necrosis factor (TNF)-alpha]. The mechanism of colorectal cancer immune evasion is multifactorial and in summary involves shift from Th1-Th2 immune responses, loss/down-regulation of human leucocyte antigen (HLA) class I antigen processing and presentation, defective dendritic cell function, T-cell loss of signalling molecules, escaping death receptors, HLA G expression, transforming growth factor (TGF) beta, vascular endothelial growth factor (VEGF), impaired natural killer (NK) activity, regulatory T-cells, and complement decay accelerating factor CD55 (18).

White blood cell composition seems to vary in colorectal cancer patients, with elevated percentages of CD8 T-cells in the initial stages, but reduced numbers of total lymphocyte count, monocyte and NK cells, IL4 and IL6 production in advance tumour stage as well as reduced levels of IFN gamma, TNF-alpha seen in vascular invasion. This shift in cytokine balance has been observed by many others (16, 20-21). TGF-beta regulates cell proliferation, differentiation, adhesion, apoptosis and angiogenesis (22) and levels correlate with Duke's staging (23). Notably TGF-beta inhibits immunotherapeutic agents including vaccines (24).

IFN-gamma, TNF-alpha and IL2 are key components of cell-mediated immunity and released by Th1 $CD4^+$ T-cells. In contrast IL4, IL6 and IL10 play a major role in humoural mediated immunity (25), through Th2 $CD4^+$ lymphocytes. Th1 activation and in turn cytotoxic T-cell, NK, macrophage and monocyte activation may result in tumour rejection, whereas Th2 activation causes the opposite effect (26). In colorectal cancer helper T-cell responses are impaired; reduced concentrations of Th1 $CD4^+$ and related cytokines (IFN-gamma, TNF-alpha, IL2) have been found in colorectal patients, levels of which correlate with disease severity (27) but also normal or elevated numbers of Th2 $CD4^+$ cells (16, 28). More advanced disease is associated with more significant imbalance in Th1/Th2 favouring Th2 responses (29-31). Low $CD4^+/CD8^+$ ratio has been associated with a better clinical course and 5-year survival (8).

The exact mechanism of shift in immune response from Th1 to Th2 is still unclear and is likely to involve chronic inflammatory changes (32) but overexpression of cyclo-oxygenase 2 (COX-2) (33-36), histamine (37-40) and IL10 (17, 41-11) have all been implicated as affecting angiogenesis, cell apoptosis, modulation of immune response and hence tumourigenesis.

Endogenous tumour synthesis and up-regulation of histamine and histidine decarboxylase causes immuno-suppression, again inhibiting Th1 cytokines (38) and cell-mediated immunity (45) and correlating with tumour stage (46). COX-2 has also been implicated in colorectal cancer carcinogenesis by inhibiting apoptosis, increasing angiogenesis and invasiveness, converting pro-carcinogens to carcinogens and modulating inflammation and immuno-suppression as well as production of Th2 cytokines which inhibit synthesis of Th1 cytokines (35). IL10 is overexpressed in colorectal cancer (30) and may inhibit Th1 cytokine production as well as antigen presentation to Th1 cells (41). The level of IL10 serves as a negative prognostic factor for treatment response as well as survival (47). Further research has highlighted the importance of p53-specific Th1 cells for improved tumour infiltration. The tumour antigen p53 is commonly mutated and overexpressed in colorectal cancer and p53-specific T-helper cells have been identified as lacking cytokines such as IFN-gamma, TNF-alpha, IL4, IL5, or IL10 (48). In contrast, tumours in patients with p53-specific IFN-gamma-producing Th cell immunity were associated with better leukocyte infiltration.

The Th1 hypothesis was also supported in a recent study by Burgdorf *et al*. in which increasing levels of pro-inflammatory cytokines such as plasma GM-CSF, TNF-alpha, IFN-gamma, IL2 and IL5 were detected in patients with stable disease after dendritic cell vaccination (49). This suggests that vaccine induced Th1 responses will be important for efficacy of colorectal cancer vaccines. Research into Th1 associated genes has shown that patients with up-regulation of Th1-related genes had a better prognosis possibly because intra-tumoural T-cells can modify cancer cells and attenuate their metastatic potential.

HLA I antigens also play a key role in tumour immunology as they present tumour associated antigen-peptides to cytotoxic T lymphocytes (50). While it has been reported that some colorectal tumours lack the HLA-ABC antigens, Diederichsen *et al*. showed that some tumours were in fact weakly positive for HLA-ABC (51). They went on to examine the pattern of HLA expression on tumour cells and concluded that HLA I expression correlated with lymphoid infiltration, showing that colon cancer cells had the functional ability of an immune response but were incapable of initiating it because of concomitant minimal HLA II

expression and absence of other co-stimulatory molecules (52) causing inactivation of infiltrating lymphocytes. HLA class I expression is altered in colorectal cancer cells as a result of mutations in the genes (53, 54) and leads to either loss or down-regulation of their processing and presentation function and subsequent tumour cells escape from immune surveillance (55-57). If indeed tumour cells are unable to initiate an immune response, stimulation of the immune system can occur by using dendritic cells (DCs) loaded with tumour antigen *in vitro* and returned to the host.

Most tumours express exclusively MHC class I, making them amenable to $CD8^+$ T-cell recognition and in fact vaccination procedures involving adoptive transfer of activated antitumour CTLs have been successful (58-59). However, it is activation of tumour-specific $CD4^+$ Th cells that is likely to confer long-lasting vaccination against tumours, as has been shown in studies after vaccination with peptides containing tumour-derived $CD4^+$ Th determinants (60). In addition, CD4-knock-out mice were shown not to be protected after vaccination (61, 62).

Apart from HLA expression for antigen recognition, DCs are also crucial in antigen recognition and presentation to other immune cells. Colorectal cancer patients also observed to have impaired DC activation within the tumour and to have reduced numbers of peripheral DC and altered T-cell stimulatory capability as well (63). Cancer cells produce immune suppressive factors (VEGF, IL10, PGE 2), which disable DC differentiation, maturation, migration and function, hence interfering with the whole adaptive immune cascade (64-66). Tumour-specific T-cell responses are further inhibited by increase in pre-cursor immature myeloid cells, which are immunosuppressive (67) and inhibit T-cell proliferation and tumour-specific T-cell response (68). A large number of non-functional immature dendritic cells are thought to be produced by altered haematopoiesis caused by tumours (69). In colorectal cancer, loss of adenomatous polyposis coli (*APC*) tumour suppressor gene has been associated with ineffective haematopoiesis, allowing hematopoietic progenitor cells to enter the cell cycle, leading to exhaustion of the myeloid progenitor pool (70). DC infiltration is also associated with improved survival (71), whereas suppression has been linked to colorectal cancer metastasis (13, 72).

Regulatory T-cells ($CD4^+CD25^+$ Treg cells), also known as suppressor cells, are a subpopulation of T-cells that act to suppress activation of the immune system and thereby maintain immune system homeostasis and tolerance to self-antigens. They also play a role in suppression of tumour associated antigen (TAA) immunity (73) through release of immunosuppressive cytokines, such as IL10 and TGF-beta (74) and inhibition of antigen-specific $CD4^+$ and $CD8^+$ cytokine production. In addition they inhibit NK and DC functions (76, 77) and are widely present in colorectal cancer patients (76). In contrast, regulatory T-cell suppression increases the efficiency of the antitumour immunity (78) and perhaps their manipulation in conjunction with other therapies may lead to better response to treatment. In a phase II trial with FOLFOX-4 (oxaliplatin, leucovorin and 5-fluorouracil) and granulocyte macrophage colony-stimulating factor and low dose IL2 in colon cancer patients, high objective response and disease control rates were observed in the treatment group with significant reduction in Treg cells (79). Depletion of Treg cells has also been shown to specifically enhance antigen-specific immune responses to cancer vaccines in patients with CEA expressing malignancies (80). Apart from abnormal T-cell function, colorectal cancer patients carry atypical T-cell receptors (TCR) with down-regulation of key signalling molecules (81) such as CD3-z (82) and $p56^{lck}$ (83) and hence impaired cytotoxic activity.

In summary, colon cancer cells need to interact synergistically with their microenvironment in order to expand and metastasize. Colorectal tumours usually grow in immunocompetent hosts, by evasion of recognition and elimination by the immune system. The research presented shows that colorectal tumours are often infiltrated by lymphocytes that are themselves activated, leading to the assumption that tumours evoke an immune response that may not be sufficient to prevent tumour growth. The tumour microenvironment is complex and capable of suppressive effects in the immune system, such as HLA loss, explaining why sometimes therapy is unsuccessful but the human system is capable of limiting tumour growth to some extent.

These data support the need to develop vaccines that trigger $CD8^+$ cytotoxic T-cell lymphocyte (CTL) responses and Th1 helper immune responses and to limit the secretion of inhibitory cytokines or function of inhibitory cell populations.

## Colon Cancer Vaccines

Although the concept of a preventative vaccine is appealing, current colorectal cancer vaccines are applied to activate the immune system to destroy tumours once they are detectable and, therefore, are considered 'therapeutic'. Furthermore, vaccines are considered 'active' immunotherapy, that is, they activate an immune response, rather than 'passive' immunity, which occurs when immune effectors such as antibodies are administered.

The identification of tumour antigens led to a notable range of approaches for generation of cancer vaccines such as: T-cell epitope peptides, defined carbohydrates of glycoproteins and glycolipids, antibody-based anti-idiotype vaccines, plasmid DNA and recombinant viral vector vaccines, allogeneic or autologous whole tumour cell vaccines, dendritic cell-based vaccines, oncolysates or

autologous heat-shock protein-peptide complex vaccines. Colorectal cancer vaccines, like other tumour vaccines are designed to either enhance anticancer immune responses (active specific immunotherapy) or to administer immune effectors to patients (passive immunotherapy-adaptive cellular immunotherapy), often in combination with an immunomodulating agent in an attempt to activate host immune response and especially T-cell-mediated immunity against tumour associated antigens. Viral vectors, DNA, proteins, peptides, recombinant viruses, anti-idiotype antibodies, have all been used among other mediators for active specific immunotherapy. Molecular modification techniques can be used to alter tumours to express co-stimulators or cytokines to stimulate T-cells. Such 'autologous' tumour cells vaccines may have therapeutic potential especially as preliminary studies showed improved recurrence free interval and survival (84).

## Tumour Antigens in Colorectal Cancer

The identification of tumour-associated antigens (TAA), whether products of mutated oncogenes/tumour suppressor genes, aberrantly expressed cellular proteins, oncofetal antigens, altered cell surface glycolipids and glycoproteins or cell type-specific differentiation antigens, has formed the basis of various approach to anticancer vaccination, as TAAs tend be present in small quantities on normal cells, if at all, and they are in theory capable of triggering an immune response. For vaccination to be effective, these TAAs need to be recognised by the immune system and hence the mechanisms of antigen processing and presentation in cancer cells need to be understood.

Given that tumours arise from uncontrolled growth of normal self cells, the majority of tumour associated antigens closely resemble self antigens. As T-cells undergo thymic selection the majority of T-cells able to recognise self-antigens are deleted as part of a central tolerance mechanism, leaving behind a smaller number of T-cells with weak affinity for self major histocompatibility antigens that would also be able to recognise 'self' tumour antigens (85). However, a limited number of TAAs do exist which are unique to the tumour and have never been presented in the thymus, and consequently the immune system recognises them as 'foreign'.

p53 is a nuclear protein, which normally mediates cell cycle arrest; its mutation leads to uncontrolled cell growth and tumourigenesis. It serves as an antigen of choice for the study of immune response to TAAs and hence the design of anti-cancer vaccines. Studies have demonstrated that p53 has antigenic properties that may activate T-cells and enhance antitumour immunity (86-88). In actual fact, vaccination with p53-specific CTLs has conferred some tumour protection in mice (89-90), regardless of tumour origin (91-92) but the reason why the response is ineffective in non-vaccinated individuals remains unknown. Different mechanisms of the mutated and wildtype antigen processing and presentation in normal and cancer cells have been implicated. Wild-type p53 self-peptides, because of their relatively confined expression in the cell nucleus, are unlikely to be presented on haematopoietic cells in context with MHC class II. In contrast, p53 expression in neonates is abundant in the thymus and probably leads to processing and presentation by MHC and thymic selection for self-p53-reactive T-cells (93). Anti-p53 CTL responses in normal and p53 knock-out mice have been investigated. In fact some wild-type p53 peptides are presented in MHC class I during development and mediate negative selection of corresponding p53-reactive $CD8^+$ T-cells (94). At the same time some peptides, even though bound to MHC class I molecules, failed to induce negative selection and vaccination with those peptides promoted antitumour immunity but not auto-immunity leading to speculation that it is possible that self p53 peptides might have an influence on $CD4^+$ T-cells as has been demonstrated (95). These findings indicate that some antigens may induce tolerance in only part of the immune system, a fact which can be manipulated in the design of vaccines eliciting responses against distinct antigenic peptides.

*βHCG*. In contrast to other oncofoetal antigens, beta human chorionic gonadotrophin (βHCG) is not produced by normal colorectal cells (96-99). βHCG expression in colorectal cancer is thought to lead to an increase in tumour invasiveness, higher metastatic incidence and promotion of tumour growth as well as promote neovascularisation and suppress the immune system (52). Its presence is thus linked to reduced survival and hence immunisation against βHCG is appealing as it might result in humoural and cellular immunity directed against HCG expressing tumour cells.

*CEA*. Carcinoembryonic antigen (CEA) is an oncofetal antigen that when expressed within recombinant poxviruses has shown induction of HLA-restricted, CEA-specific T-cells (100), suggesting that CEA can serve as a target for vaccine development (101). CEA is abundantly present on the majority of colon tumours and relatively absent from normal tissue. It would thus serve as an ideal target to destroy cancer cells without impacting on non cancer cells. However CEA is usually present in foetal development and the immune system shows some degree of tolerance from previous sensitization. Anti-idiotypes were thus created, using molecular modification techniques to create an artificial protein and attach it to the CEA idiotype, creating a new protein, sufficiently different from CEA that the immune system would not identify it as normal body protein, yet similar enough to CEA that the immune system attacks both, killing cancer cells in the process.

*5T4*. 5T4 is a human oncofetal antigen (leucine-rich membrane glycoprotein) highly expressed on placental trophoblasts during foetal development but usually absent from healthy tissue (102). It is widely present in cancers such as colorectal, gastric, renal and ovarian (103-105) and its presence has been correlated with poor survival (106) possibly secondary to the enhancement of metastatic potential (107, 108).

*EGFR*. Epidermal growth factor receptor (EGFR) is overexpressed in colorectal cancer and is associated with pour outcome (109, 110). It is a transmembrane glycoprotein composed of an intracellular tyrosine-kinase domain, a transmembrane lipophilic segment and extracellular ligand binding domain, receptor for EGF and TGF-alpha. It belongs to the EGFR family of receptors or ErbB, which comprises four proteins encoded by the *c-erb B* proto-oncogene, including EGFR (ErbB1), HER2/neu (ErbB2), HER3 (ERbB3) and HER4 (ErbB4) (111).

The *HER-2/neu* proto-oncogene encodes a transmembrane glycoprotein, similar to the epidermal growth factor receptor, whose cytoplasmic domain has tyrosine kinase activity (112). HER-2/neu is overexpressed on many adenocarcinomas including those of breast and ovary and is associated with poor prognosis (113). It is also overexpressed in 20-50% of colon tumours (114, 115).

*Gastrin*. Gastrin is a hormone that stimulates gastric acid secretion (116). In colon cancer tumours, gastrin precursors are mainly expressed, which act as growth factors. These precursors represent 90-100% of the gastrin peptides produced by colon tumours, are present early in carcinogenesis and are generally absent from healthy tissue (117). Gastrin precursors can also contribute in the angiogenesis process by stimulating the expression of VEGF (118). VEGF is one of the commonest angiogenic factors released by tumour cells and high levels have been correlated with poor prognosis in colorectal tumours (119). Therefore, gastrin inhibition would in theory result in inhibition of cell growth, proliferation and metastasis

## Active Specific Immunotherapy (ASI) Vaccines

I. *Autologous Tumour Cell-derived Vaccines (Table I)*. Autologous tumour vaccines are produced by isolating tumour cells from an individual and processing these into a vaccine *in vitro*. Autologous vaccines are usually combined with an immunostimulant and can thus elicit a cytotoxic immune response to cell-surface expressed TAAs.

The oldest immunotherapies used autologous tumour cells that were irradiated or lysed and injected intracutaneously along with bacille Calmette-Guerin (BCG) or bacterial cell wall products to produce an immune response against antigens within the tumour vaccine. Isolated administration of adjuvants resulted in non specific immune stimulation and there were no long-term benefits in the treatment of colorectal cancer. The difficulties in manufacturing autologous tumour cell vaccines resulted in a scarcity of randomised clinical trials. However immunisation with specific vaccines and non specific immune adjuvants resulted in overall prolongation of disease-free (DFS) and overall survival (OS) (120-122).

Gray *et al*. were amongst the first to carry out a clinical trial of adjuvant immunotherapy with BCG and neurami-nidase-treated autologous tumour cells in stage B and C colorectal cancer in 1989 (123). This trial, as well as other similar trials, such as the Melbourne trial (124) using combined non-specific and specific immunotherapy, failed to show any difference in DFS or OS between treatment arms. ASI using autologous tumour cells with an immuno-modulating adjuvant BCG vaccine became known as OncoVAX. Hoover *et al*. carried out a randomised clinical trial with irradiated autologous tumour cells and BCG *versus* surgery alone with significant OS and DFS for the vaccinated arm (84). However a subsequent study by the Eastern Cooperative Oncology Group (ECOG) showed no significant differences between the treatment arms. Of note, the vaccines used in the ECOG trial were manufactured at different sites, leading to discrepancies across sites in the quality of the vaccine. Subgroup analysis however of the patients that were vaccinated with the standardised criteria for the manufacture of the vaccine, showed significant improvement in OS. The importance of standardised criteria for vaccine manufacture was further illustrated in a phase III study by Vermorken *et al*. where disease recurrence was significantly reduced in the vaccinated arm (120). In this large phase III study published in the Lancet, although there was no significant benefit of OncoVAX immunisation in operated stage III cancer there were differences for stage II cancer; 254 treated patients also demonstrated a statistically significant 33% increase in 5-year OS ($p$-value of 0.014) and an 80% reduction tumour progression rate at 18 months following treatment with OncoVAX, but there were no prognostic benefits in stage III patients. This may signify that vaccination settings might have to be reviewed to include patients with earlier stage disease. In fact, the US FDA allowed Intracel to proceed with a phase III trial in stage II colon cancer patients for confirmation. The first results as published in the Lancet and in Vaccine showed statistically significant 5-year OS rate and increased recurrence-free survival in stage II patients (122, 125).

Apart from live-irradiated tumour cells or lysates, virus-infected irradiated tumour cells have also been used for the creation of autologous colon cancer vaccines. Autologous tumour vaccine-Newcastle disease virus (ATV-NDV) is an autologous tumour vaccine modified by infection with the Newcastle disease virus. Ockert *et al*. compared ATV-NVD-

Table I. *Autologous tumour cell vaccines.*

| Vaccine type | Trial | Results | Reference |
|---|---|---|---|
| Autologous tumour cells &BCG | Phase III RCT Adjuvant to resection | No statistically significant differences in OS or DFS between the treatment arms. Cohort analysis: significant improvement in OS ($p$=0.02, HR 3.97) and DFS ($p$=0.039, HR 2.67) in colon cancer patients receiving ASI. | Hoover *et al.* (1993) (84) |
| Autologous tumour cells &BCG | Phase III RCT Adjuvant to resection | 44% (95% CI 7-66) RR for recurrence in RF period in all patients receiving ASI ($p$=0.023) as adjuvant to resection in stage II and III colon cancer. Larger impact of ASI in stage II disease, with significantly longer RF interval ($p$=0.011) and 61% RR for recurrences, longer RF (42% risk reduction for recurrence or death, $p$=0.032) and a trend towards improved OS. | Vermorken *et al.* (1999) (120) |
| Autologous tumour cells &BCG | Phase III RCT Adjuvant to resection | No statistically significant differences in clinical outcomes between the treatment arms. Treatment compliance with effective immunization results in DFS ($p$=0.078) and OS ($p$=0.12) trends in favour of ASI. | Harris *et al.* (2000) (121) |
| Autologous tumour cells &BCG | Phase III RCT Adjuvant to resection | OS 65% in ASI group. Significant benefits in stage II colon cancer. | Hanna *et al.* (2001) (125) |
| Autologous tumour cells & BCG (OncoVAX) | Phase III RCT Adjuvant to resection | Effect of OncoVAX as an adjuvant is statistically significant for RF interval (57.1% RRR), OS, RF survival in stage II colon cancer patients. No statistically significant prognostic benefits in stage III patients. | Uyl-de Groot CA (2005) (122) |
| Autologous tumour cells & virus ATV-NDV | Phase III RCT Adjuvant to resection | No difference in the OS, DFS and metastases-free survival between treatment arms. Subgroup analysis: significant advantage for vaccinated colon cancer patients: OS [HR: 3.3; 95%, (CI): 1.0-10.4; $p$= 0.042] and metastases-free survival (HR: 2.7; 95%, CI: 1.0-7.4; $p$=0.047) in the ITT analysis | Schulze *et al.* (2009) (129) |
| Autologous tumour cells & virus ATV-NDV | Phase II RCT Adjuvant to resection | 2-Year survival rate 97% compared to ATV/BCG (66.7%). | Ockert *et al.* (1996) (126) |
| Autologous tumour cells & virus ATV-NDV | Phase II RCT Adjuvant to resection | At 18 months, 61% of the vaccinated arm developed recurrence compared to 87% in the other arm. 40% Increased DTH reactivity in the vaccine arm. | Schlag *et al.* (1992) (127) |
| Autologous tumour cells & BCG | Phase II RCT Adjuvant to chemotherapy | ASI induced antitumour immune response is only minimally impaired by consecutive chemotherapy. Combined treatment of stage III patients with ASI and chemotherapy (5-FU/leucovorin) | Baars *et al.* (2002) (217) |

treated patients to those treated with ATV/BCG, in patients with resected colorectal carcinoma, demonstrating 97.9% survival rate after 2 years in the ATV-NDV arm compared to 66.7% in the other arm (126). Similar differences have been reported elsewhere (127, 128). In the majority these were small studies, which did however show promise for the NDV vaccine. A randomised controlled phase II/III study soon showed that NVD vaccination lead to improved OS and progression-free survival (PFS) for colon cancer patients with liver metastases but no benefit in rectal cancer (129). NDV infection of tumour cells seems to lead to improved interaction between tumour cells and T-cells and increase T-cell co-stimulatory potential.

Autologous vaccines are highly likely to contain any tumour associated antigen capable of eliciting an antitumour immune response but these are usually present in small numbers. In addition, autologous cancer vaccine development in colon cancer is extremely difficult as the primary tumour is located in a bacteria rich environment unsuitable for harvesting autologous vaccines and most metastases are located in the liver and other sites, thus very difficult to access without considerable risks. This has suggested that allogeneic tumour vaccines could be utilized, however, apart from a single study of Canvaxin outside of its intended use for melanoma (130), there are few data for allogeneic tumour vaccines in colorectal cancer. Another complexity of using tumour cell vaccines is the possibility that the re-injected tumour, despite being rendered unable to grow, still might secrete cytokines that have immunoinhibitory activity. Finally, autologous vaccination strategies require immunomonitoring, which is challenging without knowing which tumour antigens are recognised by the immune system.

II. *Colon Cancer Vaccines Based on Defined TAAs*. In order to avoid the large amount of material in autologous tumours that may be non-immunogenic, defined TAAs, deliverable in

larger amounts, or modified to enhance immunogenicity are delivered for induction of anti-TAA antibodies or T-cell mediated antitumour immune responses.

*Vaccines based on defined TAAs: Peptides and Viruses (Table II)*. TAA identification prompted the development of strategies for antitumour vaccination, in an effort to induce specific recognition of the TAA and to elicit memory so that residual tumour can be eliminated and relapse prevented. This can be achieved by generating and persisting populations of T- and B-cells that specifically recognise and react to the TAA.

Various immunomodulators such as bacterial products, cytokines, chemokines and monoclonal antibodies have been used to trigger co-stimulatory receptors and increase efficacy.

Viral vaccines based on defined TTAs, use a viral vector system to deliver the TAA and sensitive the immune response. These vaccine strategies were developed in an effort to enhance activation of T-cells following tumour antigen presentation using viral vectors as well as other techniques, such as GM-CSF to enhance recruitment of dendritic cells to the vaccination site.

*βHCG:* In colon cancer, βHCG (β human chorionic gonadotrophin) peptide vaccine, CTP37-DT (Avicine) has been used in metastatic colorectal cancer. Phase II trials showed no significant difference between the ASI and control group, but did show that antibody responses were associated with improved survival (131). In addition, CTP37-DT generated a humoural immune response against HCG protein in 73% of the patients in the study, but cellular responses were not measured.

CDX 1307 is a fusion between βHCG and an antibody against the mannose receptor currently under investigation in an ongoing Phase I trial. There is currently a non-randomised open label study taking place of CDX-1307, a mannose receptor-targeted HCG-β vaccine, in patients with incurable locally advanced or metastatic colorectal cancer among others by Celldex therapeutics (132). Interim results suggest that intradermal inoculation of CDX-1307 results in localisation of the beta subunit of HCG in antigen presenting cells. This leads to DC activation as well as cytotoxic T-cell activity against bHCG bearing tumour cells (133).

*p53:* As explained previously, CTL responses are limited secondary to central tolerance, but $CD4^+$ T-cell responses seem unaffected. Adoptively transferred p53-specific $CD4^+$ Th cells supported the antitumor response against p53-overexpressing tumours (134). Synthetic long peptide (SLP) vaccines have been shown to be immunogenic in cancer patients. SLP is a p53 synthetic long peptide and can be used to inoculate colorectal cancer patients. The p53-SLP vaccine was shown to induce p53-specific T-cell responses, the majority being p53-specific $CD4^+$ Th cell, in patients with colorectal cancer in a phase I/II trial (135). Further research is underway to improve the polarisation of the T-cell response induced. Viral vector strategies have also been used as antigen delivery systems to immunise patients against p53, including adenovirus and recombinant canary poxvirus, eliciting p53-specific T-cell responses in some patients but limited by anti-vector immunity (136-139). Finally p53 has also been developed into dendritic cell vaccines, where the latter are pulsed with known p53 HLA-A2.1-inding peptides, resulting in induction of T-cell responses in some of the treated patients (140).

*EGFR2: EGFR* mutations can enhance tyrosine kinase activity in response to EGF and increase the efficacy of anti-EGFR such as gefitinib or erlotinib (141, 142) and hence a lot of research has focused on EGFR inhibitors. It can also serve as a target TAA for vaccination. In fact it has been shown that epitope-specific immunisation is feasible for active anti-EGFR immunotherapy (143). A recently developed vaccine used two chimeric, human epidermal growth factor receptor (HER2) B-cell epitopes fused to a promiscuous T-cell epitope and nor-muramyl-dipeptide as adjuvant. In a phase I trial, the vaccine elicited antibody responses in a subset of patients (144).

*Gastrin:* G17DT (Gastroimmune) is an antigastrin-17 immunogen, raising antibodies that blockade gastrin-stimulated tumour growth (145). Therapeutic efficacy of immunisation with G17DT has been established in various tumour settings including primary and metastatic disease (146). Phase II trials with G17DT immunisation in otherwise refractory metastatic colorectal cancer showed additive effects of improved survival when combined with irinotecan chemotherapy (147, 148).

*CEA:* The majority of CEA vaccines use viruses such as ALVAC for its delivery to tissue, often in association with co-stimulatory molecules. For example, ALVAC-CEA/B7, is a vaccine based on a viral vector system derived from the non-replicating canarypox virus, which has been modified to express CEA and has been used in patients with first-line metastatic colorectal cancer. ALVAC is different to other vaccines in that it was constructed to express both CEA and the B7.1 co-stimulatory molecule to enhance the antitumour vaccine (149). B7.1 (CD80) co-stimulatory molecule addition enables binding to CD28 on the surface of T-cells leading to cell proliferation and cytokine release (150). In a combination regimen with standard chemotherapy, this vaccine resulted in increase in CEA specific T-cell responses with no difference in clinical or immune response amongst treatment groups (151). Chemotherapy was not shown to have an adverse effect on CEA-specific T-cell immunity. There are two trials in process, the first trial (pilot phase II) assesses the safety and immunologic activity of ALVAC co-administered with chemotherapy and the second trial when co-administered or following chemotherapy (phase I).

ALVAC has also been used as a vector to deliver Ep-CAM. Ep-CAM (epithelial cell adhesion molecule) is an important mediator of cell-cell interaction and influences growth, differentiation and organisation within tissues (152, 153). KSA is a human pancarcinoma antigen, a glycoprotein of 40 kDa, which is aberrantly expressed in epithelial tumours (154). KSA is thought to function as an EpCAM and it is highly expressed in colon tumours (155). The EpCAM gene is inserted into the ALVAC virus along with GM-CSF. Vaccination has been shown to induce a tumour-specific cellular immune response (156). Vaccines based on viruses such as ALVAC work through cross-presentation rather than direct stimulation of T-cells by the infected cells and it is difficult to determine whether they do indeed lead to improvement of vaccine efficacy.

Apart from ALVAC, the recombinant fowlpox virus has also been used in association with CEA to create rF-CEA(6D)-TRICOM. In this *ex-vivo* system, generated DCs are modified or infected by a recombinant fowlpox vector to hyper-express a triad of co-stimulatory molecules (TRICOM) and CEA, which are then used as a vaccine. Phase I studies showed an increase in the frequency of CEA-specific immune response among both $CD4^+$ and $CD8^+$ T-cells in all immune responders (157). Similar results were achieved with rV-CEA(6D)-TRICOM which uses vaccinia vector to prime an immune response and then boosts are given with rf-CEA(6D)-TRICOM (158).

A further extension of the CEA-TRICOM approach has been to add an additional antigen to generate PANVAC-VF. It uses both recombinant vaccinia and fowlpox to express both epithelial mucin 1 and CEA as well as co-stimulatory molecules. Early clinical trials are evaluating PANVAC alone and in combination with conventional chemotherapy and/or radiation (159). Interest in poxviruses arose as they can accommodate multiple transgenes. In a pilot study, patients were vaccinated with recombinant vaccinia for CEA, MUC-1 and TRICOM (PANVAC-V) followed by fowlpox (PANVAC-F) as a booster eliciting anti MUC-1 and/or anti-CEA responses through both CD8 and CD4 cells and early evidence of clinical benefit (160). An ongoing study is testing the role of PANVAC-VF in patients with resected hepatic metastases of colorectal cancer (NCT00103142).

*5T4*: TroVax uses a tumour-associated antigen, the human oncofetal antigen 5T4 with a pox virus vector, the modified vaccinia virus Ankara (MVA). Pre-clinical murine models had suggested that TroVax resulted in as high as 90% reduction of tumour burden (161). Antitumour activity and protection was shown to be highly dependent on $CD4^+$ T-cells and antibody mediated. However murine models using CEA $CD8^+$ cells showed that $CD8^+$ T-cells were essential for protection without a role in destruction of tumour cells and CEA antibodies had no role in tumour destruction (162). These murine models also suggested preventative on top of therapeutic benefits, even though in mice models it is often easier to protect mice against tumours in a preventative setting. Mice injected with MVA expressing murine 5T4 resulted in induction of immune response against m5T4 such that when challenged with syngeneic tumours expressing murine 5T4 they were protected (163). In man, antibody-mediated effector mechanisms would not be affected by MHC class I down-regulation in tumour cells and hence induction of 5T4-specific antibody response would be ideal for targeting membrane antigens. The first human phase I/II trial showed the vaccine to be safe and well tolerated inducing 5T4-specific immune responses in 94% of the 22 participants. There was no correlation between enhanced patient survival and MVA-specific immune responses. Overall, 41% patients showed disease stabilisation (163).

*Vaccines based on defined TAAs: Anti-idiotype antibodies (table III).* Anti-idiotype antibodies mimic TAAs. When the binding site of an antibody (idiotype) is recognised by the immune system as foreign, a new antibody against the binding site is created, which is known as the anti-idiotype. The latter resembles the original antigen. The peptides of the anti-idiotype's binding site closely resemble the original antigen peptide structure and can also be processed and presented to T-cells resulting in the formation of antigen specific T-cell responses (165).

CeaVac (Titan Pharmaceuticals) is a vaccine based on anti-idiotypic antibodies and mimics CEA. Initially, in a phase II study (n=32) with patients with minimal residual disease, all vaccinated patients were shown to have a high titre of IgG and T-cell immune response against CEA (166). In a larger phase III trial (n=631), CeaVac failed to show any improvement compared to placebo and 5-fluorouracil and leucovorin (167). Subset analysis showed correlation between OS and number of injections. Recently, in a phase II prospective multi-institutional trial, patients with curatively resected colorectal cancer hepatic metastases received CeaVac in combination with TriAb (human milk fat globule) as an adjuvant but vaccination did not improve 2-year recurrence-free survival when compared with the expected value of 40% reported for hepatic resection alone (81).

Another similar vaccine is Onyvax-105 which uses TAA 105AD7, which is another anti-idiotype antibody what mimics CD55 (791Tgp72) (169). CD55 is a glycosyl-phosphatidylinositol-anchored protein that regulates complement activation (170) and inhibits the formation of membrane attack complex (171) protecting cells against complement attack. Complement attack is a powerful mechanism in the immune defence against cancer (172). Colorectal cancer cells express a broad spectrum of CD55 to escape from complement attack (173). Tumour cell lysis may occur after vaccination as the vaccine is thought to stimulate host-cytotoxic T-cell response against tumour cells

Table II. *Peptide and viral vaccines*.

| Vaccine type | Trial | Results | Reference |
|---|---|---|---|
| CTP37-DT Avicine Peptide vaccine with BCG | Phase II RCT | Vaccination with CTP37-DT induced anti-hCG antibodies in most patients with advanced colorectal cancer. Anti-hCG antibody induction was associated with longer overall survival ($p$=0.0002). | Moulton (2002) (131) |
| G17DT Gastroimmune Peptide vaccine with diphtheria | Phase II adjuvant to chemotherapy | N/A | Gilliam (2007) (145) |
| G17DT Peptide vaccine | Phase II Combination chemotherapy | N/A | Rocha Lima *et al*. (2004) (147) |
| CDX-1307 | Phase I | Ongoing | Morse (2009) (133) |
| P53 Synthetic long peptide vaccine | Phase I/II | p53-specific T-cell responses were induced in 9 of 10 colorectal cancer patients. In 6/9 tested patients, p53-specific T-cell reactivity persisted at least 6 months. p53-specific T-cells isolated from the vaccination site were characterized as $CD4^+$ T-cells producing both T-helper types 1 and 2 cytokines. | Speetjens *et al*. (2009) (135) |
| P53 Recombinant canarypox/ALVAC | Phase I/II | T-cell and IgG antibody responses against the vector component of the ALVAC vaccine were induced in the majority of the patients. immunosorbent-spot assay (ELISPOT) analysis of vaccine-induced immunity revealed the presence of IFN-gamma-secreting T-cells against both ALVAC and p53. | van der Burg *et al*. (2002) (138) |
| PanVacVF Recombinant fowlpox & Vaccinia CEA/MUC-1 | Phase I | PANVAC-VF is safe and is associated with the generation of CD8 and CD4 antigen-specific immune responses post vaccination. These immune responses were seen in more than half of patients tested. | Gulley (2008) (160) |
| ALVAC-CEA/B7.1 Recombinant Canarypox virus/CEA | Phase II RCT Combination chemotherapy | 40.4% Objective clinical responses. All patients developed antibody responses against ALVAC; Increase in CEA-specific T-cells as high as 50% in ALVAC with chemotherapy and booster. No differences in clinical or immune responses between the treatment groups. Chemotherapy did not affect the generation of CEA-specific T-cell responses following vaccination. | Kaufman (2008) (151) |
| ALVAC-KSA Recombinant canarypox virus/ Ep-CAM/KSA and GM-CSF | Phase II RCT | ALVAC-KSA, in combination with low dose local administration of GM-CSF may induce a strong, IFN-gamma T-cell response. | Ullenhag (2003) (156) |
| ALVAC-p53 Recombinant canarypox virus & tumour associated auto-antigen p53 | Phase I-II escalation RCT | Potent T-cell and IgG antibody responses against the vector component of the ALVAC vaccine were induced in the majority of the patients. | Van der Burg (2002) (138) |
| rF-CEA(6D)-TRICOM fowlpox vector to infect dendritic cells & co-stimulatory molecule | Phase I RCT | Increase in the frequency of CEA-specific T-cells in 10 patients. Cytokine flow cytometry showed CEA-specific immune response among both $CD4^+$ and $CD8^+$ T-cells in all immune responders. Safe and activates potent CEA-specific immune responses. | Morse (2005) (187) Marshall |
| rv-CEA(6D)-TRICOM vaccinia vector to infect dendritic cells & co-stimulatory | Phase I RCT | CEA-specific T-cell responses were observed in the majority of patients tested. | (2005) (158) |
| 5T4-TroVax Pox virus vector-MVA | Phase I/II | 5T3-Specific antibody response generated in 94% of 22 vaccinated. No correlation between enhanced survival and MVA specific esponses. 41% showed disease stabilisation. | Harrop *et al*. (2006) (161) |

Table III. *Anti-idiotype vaccines*.

| Vaccine type | Trial | Results | Reference |
|---|---|---|---|
| CeaVac<br>Anti-idiotype<br>monoclonal antibody | Phase II RCT | Vaccine as adjuvant to chemotherapy and vaccine alone. CeaVac consistently generated a potent anti-CEA humoural and cellular immune response in all patients entered into this trial. 5-FU regimens: no effect on immune response. | Foon (1999) (166) |
| CeaVac & Polyab<br>Anti-idiotype<br>monoclonal antibody | Phase II prospective<br>Adjuvant to resection | CeaVac and human milk fat globule (TriAb).<br>Vaccine therapy of colorectal cancer hepatic metastases did not improve 2-year RF survival (39%) when compared with the expected value of 40% reported for hepatic resection alone. | Posner (2008) (168) |
| 105AD7 (OnyVac)<br>Anti-idiotype | Phase II RCT<br>Neo-adjuvant/adjuvant | Immune responses to vaccination were induced in a majority of monitored patients. RF survival, OS: NR. | Ullenhag (2006) (177) |
| 105AD7(Onyvax)<br>Anti-idiotype<br>Monoclonal antibody | Phase II RCT | 105AD7 Vaccination does not prolong survival in patients with advanced colorectal cancer. | Maxwell-Armstrong (1999) (174) |
| SVC106<br>Anti-idiotype | Phase II RCT | Vaccination of immunologically responding metastatic colorectal carcinoma patients with SCV 106 leads to slowed disease progression and tumour dissemination and significantly prolongs survival time. | Samonigg (1999) (178) |
| 5T3 TroVax<br>Anti-idiotype<br>MVA | Phase I/II | 5T4-Specific immune responses in 94% of the 22 participants.<br>No correlation between enhanced patient survival and MVA-specific immune responses. Overall, 41% patients showed disease stabilisation. | Harrop (2006) (161, 164) |
| 5t3 TroVax | Phase II<br>Adjuvant to chemotherapy | 88% showed positive response for the 5T4 antigen and 95% for the vector system (MVA). Significant associations between immune responses and OS across trials. RR 14% ($p<0.01$) and 13% ($p=0.01$). In addition, combination of two treatment modalities did not enhance toxicity and was in fact shown to induce potent 5T4 immune responses. | Harrop (2007) (209) |

expressing CD55. Case-control studies on patients with minimal residual disease suggest there might be a slight survival advantage (175). Several randomised control trials (neo-adjuvant/adjuvant) failed to show improvement in survival (176, 177) and no further research has been conducted since.

SCV106 is another anti-idiotype, this time goat antibody, vaccine. It mimics the 17-1A glycoprotein antigen associated with colorectal cancer. In a double blind randomised phase II trial overall prolongation of survival was shown in immunoresponders (178) compared to patient receiving unspecific goat antibodies. Twenty-nine out of 42 patients mounted an immune response. Comparison of immunological responders in both groups revealed a significant survival advantage of the SCV 106-treated patients compared with controls (mean 67 *versus* 39 weeks; $p=0.01$). No further studies were carried out.

III. *DC-based vaccines*. DCs are derived from haemopoetic cells and act as antigen-presenting cells for triggering T-cell immunity in the context of both MHC I ($CD8^+$ CTL) and II ($CD4^+$ T-cells) (179). Most DC vaccines are prepared by pulsing DCs with either tumour lysate or RNA, by transfection with tumour DNA or by creating tumour cell/DC fusions (180).

As with autologous tumour-cell derived vaccines, autologous dendritic cells have also been used as platforms for cancer vaccines and induce tumour-specific immune responses as well as objective clinical responses in colorectal cancer patients (181, 182). For example, tumour cell lysate pulsed DCs induce an antitumour T-cell response both *in vitro* and *in vivo* (183, 184). In another phase II study, autologous DCs were pulsed with allogeneic tumour cell lysate containing cancer testis antigens and administered to twenty advanced colorectal cancer patients showing median survival of 5.3 months with stable disease in 24% patients in an otherwise safe and non toxic vaccine (185).

Viral vectors have also been used to load DCs with tumour antigens or transduce them with tumour-specific genes often in association with co-stimulatory molecules to enhance the response. In colorectal cancer, CEA is the most widely used antigen for loading DCs (186). For example active immune response was generated after DCs were transfected with a fowlpox virus encoding CEA and administered to metastatic colorectal cancer patients (187). CEA-specific immune responses have also been elicited through similar vaccines, using DCs loaded with CEA peptides (188-190). Immunisation with DC cells pulsed with HLA-restricted CEA peptides resulted in increase in CEA specific T-cells in 7 out of 10 patients. Monocyte-derived DCs can also be transfected

with RNA encoding tumour antigens, for example CEA RNA to induce T-cell reactivity (191). Such antigen-specific T-cell responses can readily be detected in delayed type hypersensitivity (DTH) skin tests and correlate with outcome (192). However, it is only mature DCs that can stimulate T-cell responses (193-195). This can explain why other studies with CEA-peptide pulsed DCs did not find CEA-specific immune responses. Validated assays are also needed to monitor immunological outcome, since clinical responses occur in a minority of patients. These assays should ideally run on tumour tissue and lymph nodes rather than peripheral blood, where precursor frequencies might be low.

DC vaccines have also been used for the investigation of responses to multiple TAAs by loading autologous DCs with peptides derived from multiple TAAs to determine priming of antigen specific CD8 T-cells and whether responses to all of the antigens included in the vaccine can be raised. The vaccine was manufactured using GM-CSF and IL13 to generate dendritic cells (DCs) from monocytes. The DCs were loaded with 6HLA-A*0201-binding peptides derived, among others, from CEA, MAGE-2 (melanoma antigen overexpressed in gastrointestinal cancer (196), and *HER-2/neu*. In a phase I/II trial, inoculation with this vaccine in patients with advanced colorectal cancer resulted in induction of T-cell responses not only to CEA-derived peptides but also to multiple tumour-associated antigens (197). Sixty-five (47.4%) out of 137 patients were determined by immunohistochemistry to overexpress *HER-2/neu* protein. *Her-2/neu* gene amplification was detected in two patients by fluorescent *in situ* hybridisation (FISH) (198).

The Nargosten and Thiel meta-analysis of 527 patients with advanced colorectal cancer in 32 studies estimated the clinical and immunological responses to ASI vaccines (199). One complete response and four partial responses were observed. For DC-based vaccines alone the clinical benefit rate (the sum of complete responses, partial responses, mixed responses and stable disease rates) was 17% compared to 11.2% for all patients.

One of the main disadvantages of using DC vaccines is limitations in their availability and costly as well as laborious generation. DCs represent about 0.2% of peripheral blood leukocytes but can readily be generated from precursors in an immature form. They can then be maturated through the use of GM-CSF and IL-4 as well as other cytokines (200-203). Additionally they can be derived from CD34+ precursors in the blood or through leukapharesis, though in small numbers or *in vivo* through administration of DC growth factors. However, it has been demonstrated than *in vivo* expansion of the blood DC pool in cancer patients is feasible (204).

More research is needed into the immunisation route and influences on T-cell migration to facilitate transfer of activated cytotoxic T-cells to tumour sites as objective clinical responses remain low (205).

IV. *Combination Vaccines*. As already mentioned, chemotherapy forms the standard treatment for the majority of colorectal cancers. In advanced colorectal cancer, chemotherapeutic combination therapy appears to provide better results in terms of response rate and survival (206, 207).The combination of chemotherapy with vaccination has formed a relatively new field of research with promising results. 5-FU, folinic acid and oxaplatin have all been used in combination with vaccines in an effort to create an active antitumour 'immunochemotherapy' (208).

The realisation that colorectal cancer is immunogenic led to the exploration of immune therapy. Select chemotherapy drugs have off-target immune effects, such as increased expression of CEA with 5-FU administration, which can be used in combination with novel tools to stimulate both adaptive and innate immune mechanisms. Combination treatment is capable of inducing both growth inhibition and CEA up-regulation, making tumour cells more amenable to the cytolytic activity of the specific effector lymphocytes. Vaccine use in these settings aims at eradication of target malignant T-cells after or during chemotherapy. Prete *et al*. measured the combined effects of various chemotherapeutic agents in CEA expression in colorectal cancer patients (208). CEA protein expression was markedly up-regulated when fluoropyrimidine was administered prior to oxalipatin. This finding can be exploited with concomitant use of CEA directed vaccines as tumour cells are likely to be rendered more susceptible to the cytolytic activity of specific effector lymphocytes following vaccination.

In another recent cross-trial analysis of one phase I/II and three phase II trials in patients with metastatic colorectal cancer, where a median of five TroVax injections were administered either alone, as adjuvant to surgery or in combination with chemotherapy (FOLFOX and FOLFIRI) (209, 210). Fifty-nine patients were immunologically evaluated. Of theses 88% showed positive response for the 5T4 antigen and 95% for the vector system (MVA). Exploratory analyses showed significant associations between immune responses and overall survival across trials. In fact, doubling in the 5T4 specific antibody response after the second and third vaccination was independently associated with a reduction in relative risk of death of 14% ($p<0.01$) and 13% ($p=0.01$). In addition combination of two treatment modalities did not enhance toxicity and was in fact chemotherapy in synergy with the vaccine was shown to induce potent 5T4 immune responses.

One of few meta-analyses of active specific immunisation in colorectal cancer by Nagorsen and Thiel (2006) showed an overall response rate of 0.9%, including autologous tumour cells, peptide vaccine, dendritic cells, idiotypic antibody, and virus-based vaccine, with immune responses elicited in at least 44% of patients (199). Disease stabilisation was observed in 8.3% cases with a very poor response rate (<1%) in patients with advanced disease.

## Expert Opinion

Cancer vaccines have been under research for over two decades with efforts to develop biologically active immunotherapies against tumour cells without detrimental toxicities.

Tumour vaccine efficacy depends on host-vaccine interactions, especially in terms of immunogenicity of the cancer, the status of the host immune response (to recognition and effector mechanisms) and development of host T-cell-mediated immunity and memory. Therefore, the potency of colon cancer vaccines depends on its ability to induce host antitumour response and systemic cell mediated immunity. It is not only successful induction of host antitumour response that is necessary but manipulation of the immune system beyond the initial stimulation too.

Memory $CD8^+$ T-cells generated without $CD4^+$ T-cell help are defective in their ability to respond to secondary encounters with antigen. Naive $CD4^+$ T-cells upon *in vivo* priming differentiate into either Th1 or Th2 effector cells. IL12 and IFN-gamma can drive the differentiation towards Th1. Long-term memory T-cells maintain the phenotype induced at the time of priming. Long-term memory appears to be linked with survival in cancer. Therefore, a 'prime-boost' vaccination with a DC-based vaccine for priming and a tumour cell based vaccine for boosting may be superior, as it will target both naive and memory T-cells and may optimise an overlap with MHC.

As described earlier, the majority of tumour infiltrating lymphocytes that recognise overexpressed tumour/self tumour antigens or TAAs are actually T-cells that escaped negative thymic selection and hence their activation is suboptimal (211). Most vaccines already described aim at enhancing the function of these T-cells. Another approach would be the use of peptide variants. Also known as mimotopes, heteroclitic peptides, altered peptide ligands and superagonists, they can activate the low-affinity TAA-specific T-cells better than native antigens (212) eliciting stronger activation against native antigens (213, 214. Even though preliminary trials did not show promise (94, 215), more recently Jordan *et al*. showed that peptide-variant vaccines are most effective when the peptides react with a large responsive part of the tumour-specific T-cell repertoire (216). Evidently more research into this area is warranted.

Mechanisms of measuring antitumour immunity are also essential for monitoring vaccine efficacy. Early studies suggest that DTH reactions after autologous tumour cell vaccination correlates strongly with recurrence and cancer survival (217). There is need to develop further methods to test antitumour immunity after vaccination.

Coming on to the selection of potential vaccination targets and tumour-associated antigens, research into identification of new TAAs and their exploration is still on going. For example, human colorectal cancer antigen GA733 is a transmembrane glycoprotein and functions as a cell-cell adhesion molecule (also known as CO17-1A/KS1-4/KSA/EpCAM (218). Its extracellular domain has been used as a target in colorectal cancer vaccination trials, where recombinant protein, produced tumour specific humoural and cellular immune responses (219). In fact these responses were higher than similar responses elicited by monoclonal antibodies (220) and are even further enhanced by expression of the antigen in a viral vector, such as vaccinia (221). Recombinant chimeric protein was formed using GA733-2 fused to the Fc fragment of the antibody GA733-2-Fc and expressed from Drosophila S2 cells and was then used to vaccinate mice, eliciting specific antibody production, which bound to human colorectal carcinoma HCT-116 cells (222). The latter study was the first to report *in vivo* immunogenicity of the chimeric protein GA733-2-Fc, which may form a platform for further research and vaccine development.

There is no convincing reason to focus on any one particular antigen group more than another and, in fact, there seem to be diminishing returns from discovering more tumour antigens and it might be time to focus on discovering more platforms for tumour antigen delivery. Viruses as a vehicle of delivery of TAAs are usually attenuated and then genetically engineered to express human TAAs. The first recombinant vaccinia virus was constructed years ago and subsequently various vaccinia and other poxviruses followed. It soon became evident that molecular modification techniques also had a major role to play for successful delivery of antigens as well as optimising the tumour environment. Viruses were used to infect the tumour cells and then deliver genes for cytokines which would in turn recruit and activate antigen presenting cells at the site of vaccine injection, such as in the ALVAC-CEA/B7 trials. It becomes evident that it is impossible to talk about TAAs without mentioning delivery systems and optimisation techniques.

In addition to TAAs, we should not forget the role of growth and survival factors in the growth and spread of colon cancer which may also serve as targets for immune attack as well as timing of vaccine administration. In an ideal setting the vaccine should be administered immediately after surgery to increase the chances of eradicating micrometastases. A more recent study showed that irradiated tumour cell vaccine efficacy, measured as an increase in $CD8^+$ T-cell-mediated immunity, was increased by concomitant injection with anti-TGF beta antibodies. TGF beta inhibition has been shown to enhance antitumour immunity mediated by $CD8^+$ T-cells, acting synergistically with irradiated tumour cells (223). Approaches targeting growth factors and cytokines in combination with other modalities have not been widely explored.

Several trials demonstrated statistically significant results in phase I/II trials but subsequently failed at generating significant results in phase II or III trials, perhaps secondary

to tumour load. Such failure should lead to critical evaluation of basic concepts and of influences on scientific developments and would justify testing in earlier stage disease. Most phase I trials show safety but only a small minority of patients show objective immune response. Therefore, immunological performance status needs to be optimal and taken into account. In several trials, such as in the avicine trials, although no survival benefit was identified, subgroup analysis showed groups that actually benefitted. The need for better patient subsets may be addressed by the use of specific biomarkers for the identification of patients more likely to benefit from such immunotherapy.

In addition, it seems that the majority of cancer vaccine research focuses on patients with advanced disease. Such patients are less likely to respond as their immune systems might be compromised by the stage of disease. In contrast, promising results have been shown in testing in earlier stage disease where the immune system is likely to be more adept at identifying and destroying tumour cells. Other studies, such as the CeaVac have shown that patients who have undergone resection followed by either vaccination or no adjuvant treatment had a better PFS. Removal of the tumour might permit greater activity of the tumour vaccine against micrometastases. The majority of colon cancer vaccines are administered quite late in the disease process, sometimes even after metastatic spread, for end stage disease or after failure of other modalities. Early vaccine administration might therefore be beneficial.

Again it seems that the adjuvant setting is the most productive. However, true adjuvant cases where advanced cancer is locally resected are less common and hence large number of cases would be needed to yield significant results. However, vaccination of patients with controlled metastatic disease might be more fruitful. In a recent Cochrane review (2009) on adjuvant therapy for resected stage II colon cancer, seven adjuvant specific immunotherapy trials were identified, of which only one showed an improvement for treated patients, with 61% (18-81) risk reduction for recurrences (224) (see table I). The different results were attributed to immunogenicity, status of the immune system and development of cell mediated immunity. Significant improvement in DFS (OR: 0.75, CI: 0.56-1.00) was demonstrated in the meta-analysis of 723 patients across the four trials using autologous tumour cells and BCG, associated with an induration of more than 5 mm at the inoculation site. The results of this meta-analysis indicate that active specific immunotherapy is an important adjuvant modality for stage II patients. Therefore, adjustments in terms of patient selection, disease stages and end-points as well as combination therapies are also necessary.

There have been relatively few clinical studies to investigate the concomitant use of cancer vaccines and chemotherapy. Chemotherapy regimens have been associated immunostimulatory effects such as enhanced cross presentation of antigens, partial activation of dendritic cells promotion of long-term antigen independent memory. Therefore, combining different treatment modalities may in face provide survival benefit. Main problems include the optimum timing of delivering the vaccine relative to chemotherapy and the best chemotherapy regimen. Chemotherapy aims at reducing tumour load and intuitively this would provide an ideal setting for cancer vaccination in patients with minimal residual disease. The effect of cytotoxic chemotherapy on vaccine-induced antitumor immunity remains largely unknown.

There is also little research into the use of vaccines after tumour resection, as a tertiary prevention method, especially in patients undergoing liver resection for colorectal metastases. In phase II trials with TroVax, patients with pre-existing proliferative responses to 5T4 were the longer term survivors. Hence pre-existing 5T4 specific immune responses provide the platform for stronger 5T4 specific immune responses after TroVax (225). Even with TroVax though, additional evaluation of cellular immune responses to 5T4 in cryopreserved peripheral blood mononuclear cells from colorectal cancer liver metastasis vaccinated patients demonstrated that despite cumulative proliferative response to 5T4 immunity, more than 50% patients showed immune suppression and escape. Therefore, serological and cellular immunity are likely to manifest through direct and indirect mechanisms. Poorer survival was correlated when elevated Treg levels as a proportion of $CD4^+$ infiltrate. Manipulation of local $CD4^+$ infiltration might influence clinical outcome (226).

Combination treatments can be considered with treatment modalities other than chemotherapy, for example radiotherapy. Radiotherapy may reduce immunosuppressive factors produced at the tumour site. Hence combination strategies with vaccination may provide a solution to the ability of tumours to shield themselves from an immune attack.

The success of clinical trials of colon cancer immunotherapy may depend on other factors as well. A consensus must be reached on the most important endpoints and focus research into achievement of these. For example, it is notable that for the prostate cancer vaccine Provenge, survival was improved while PFS was not. This suggests that survival may need to be the endpoint used for some vaccines and in some clinical scenarios. Of course, this would greatly increase time to see favourable results. Also, intermediate endpoints or biomarkers of response should also be clarified.

Therefore, reliable clinical results are more likely to be obtained using patients with small tumour burden and early or minimal residual disease after chemotherapy or resection as well as assays to monitor the state of immunisation. Even though to date there is no substantial evidence that cancer vaccines are curative or result in significant improvement of

life expectancy, it is vital for research to continue, perhaps shifting into testing new delivery platforms in patients with earlier stage disease or at least in combination regimens to begin with before proceeding to identify a preventative rather than a curative vaccine which would bring a new era in colorectal cancer therapy.

## References


1 Cancer Stats Fact Sheet: Colorectal Cancer. Cancer Research UK 2007. Available at: http://publications.cancerresearchuk.org/ WebRoot/crukstoredb/CRUK_PDFs/CSFSBOWEL.pdf

2 O'Connell NJ, Mailliard JA, Kahn MJ, Macdonald JS, Haller DG, Mayer RJ and Wieand HS: Controlled trial of fluorouracil and low-dose leucovorin given for 6 months as postoperative adjuvant therapy for colon cancer. J Clin Oncol *15(1)*: 246-250, 1997.

3 Line A, Slucka Z, Stengrevics A, Silina K, Li G and Rees RC: Characterization of tumour-associated antigens in colon cancer. Cancer Immunol Immunother *51(10)*: 574-582, 2002.

4 Chan CC, Fan CW, Kuo YB, Chen YH, Chang PY, Chen KT, Hung RP and Chan EC: Multiple serological biomarkers for colorectal cancer detection. Int J Cancer *126(7)*: 1683-1690, 2010.

5 Sallusto F, Geginat J and Lanzavecchia A: Central memory and effector memory T-cell subsets: function, generation, and maintenance. Annu Rev Immunol *22*: 745-763, 2004.

6 Pagès F, Berger A, Camus M, Sanchez-Cabo F, Costes A, Molidor R, Mlecnik B, Kirilovsky A, Nilsson M, Damotte D, Meatchi T, Bruneval P, Cugnenc PH, Trajanoski Z, Fridman WH and Galon J: Effector memory T-cells, early metastasis, and survival in colorectal cancer. N Engl J Med *353(25)*: 2654-2666, 2005.

7 Baier PK, Wimmenauer S, Hirsch T, von Specht BU, von Kleist S, Keller H and Farthmann EH: Analysis of the T-cell receptor variability of tumor-infiltrating lymphocytes in colorectal carcinomas. Tumour Biol *19(3)*: 205-212, 1998.

8 Diederichsen AC, Hjelmborg JB, Christensen PB, Zeuthen J and Fenger C: Prognostic value of the $CD4^+/CD8^+$ ratio of tumour infiltrating lymphocytes in colorectal cancer and HLA-DR expression on tumour cells. Cancer Immunol Immunother *52(7)*: 423-428, 2003.

9 Naito Y, Saito K, Shiiba K, Ohuchi A, Saigenji K, Nagura H and Ohtani H: $CD8^+$ T-cells infiltrated within cancer cell nests as a prognostic factor in human colorectal cancer. Cancer Res 15 *58(16)*: 3491-3494, 1998.

10 Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pagès C, Tosolini M, Camus M, Berger A, Wind P, Zinzindohoué F, Bruneval P, Cugnenc PH, Trajanoski Z, Fridman WH and Pagès F: Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science *313(5795)*: 1960-1964, 2006.

11 Camus M, Tosolini M, Mlecnik B, Pagès F, Kirilovsky A, Berger A, Costes A, Bindea G, Charoentong P, Bruneval P, Trajanoski Z, Fridman WH and Galon J: Coordination of intratumoral immune reaction and human colorectal cancer recurrence. Cancer Res *69(6)*: 2685-2693, 2009.

12 Seino K, Kayagaki N, Okamura K and Vagita H: Antitumor effect of locally produced CD95 ligand. Nat. Med *3*: 165-170, 1997.

13 Agrawal B, Krantz MJ, Reddish MA and Longenecker BM: Cancer-associated MUC1 mucin inhibits human T-cell proliferation, which is reversible by IL-2. Nat Med *4(1)*: 43-49, 1998.

14 Merogi AJ, Marrogi AJ, Ramesh R, Robinson WR, Fermin CD and Freeman SM: Tumor-host interaction: analysis of cytokines, growth factors, and tumor-infiltrating lymphocytes in ovarian carcinomas. Hum Pathol *28(3)*: 321-331, 1997.

15 Rohrer JW and Coggin JH Jr.: CD8 T-cell clones inhibit antitumor T-cell function by secreting IL-10. J Immunol *155(12)*: 5719-5727, 1995.

16 Heriot AG, Marriott JB, Cookson S, Kumar D and Dalgleish AG: Reduction in cytokine production in colorectal cancer patients: association with stage and reversal by resection. Br J Cancer *82(5)*: 1009-1012, 2000.

17 Galizia G, Lieto E, De Vita F, Romano C, Orditura M, Castellano P, Imperatore V, Infusino S, Catalano G and Pignatelli C: Circulating levels of interleukin-10 and interleukin-6 in gastric and colon cancer patients before and after surgery: relationship with radicality and outcome. J Interferon Cytokine Res *22*: 473-482, 2002.

18 Evans C, Dalgleish AG and Kumar D: Immune suppression and colorectal cancer. Aliment Pharmacol Ther *24(8)*: 1163-1177(15), 2006.

19 Evans CF, Galustian C, Bodman-Smith M, Dalgleish A and Kumar D: The effect of colorectal cancer upon host peripheral immune cell function. Colorectal Dis 2009.

20 Elsässer-Beile U, von Kleist S, Fischer R and Mönting JS: Impaired cytokine production in whole blood cell cultures from patients with colorectal carcinomas as compared to benign colorectal tumors and controls. J Clin Lab Anal *6(5)*: 311-314, 1992.

21 Fantini MC and Pallone F: Cytokines: from gut inflammation to colorectal cancer. Curr Drug Targets *9(5)*: 375-380, 2008.

22 Kondo M, Suzuki H, Takehara K, Miyazono K and Kato M: Transforming growth factor-beta signalling is differentially inhibited by Smad2D3450E and Smad3D407E. Cancer Sci *95(1)*: 12-17, 2004.

23 Narai S, Watanabe M, Hasegawa H, Nishibori H, Endo T, Kubota T and Kitajima M: Significance of transforming growth factor beta 1 as a new tumor marker for colorectal cancer. Int J Cancer *97*: 508-511, 2002.

24 Ahmadzadeh M and Rosenberg SA: TGF-beta 1 attenuates the acquisition and expression of effector function by tumor antigen-specific human memory CD8 T-cells. J Immunol *174*: 5215-5223, 2005.

25 Dalgeish AG and O'Byrne KJ: Chronic immune activation and inflammation in the pathogenesis of AIDS and cancer. Adv Cancer Res *84*: 231-276, 2002.

26 Kidd P: Th1/Th2 balance: the hypothesis, its limitations and implications for health and disease. Altern Med Rev *8*: 223-246, 2003.

27 Nakayama H, Kitayama J, MutoT and Nagawa H: Characterization of intracellular cytokine profile of $CD4^{(+)}$ T-cells in peripheral blood and tumor-draining lymph nodes of patients with gastrointestinal cancer. Jpn J Clin Oncol *30(7)*: 301-305, 2000.

28 Pellegrini P, Berghella AM, Del Beato T, Cicia S, Adorno D and Casciani CU: Disregulation in TH1 and Th2 subsets of $CD4^+$ T-cells in peripheral blood of colorectal cancer patients

and involvement in cancer establishment and progression. Cancer Immunol Immunother *42*: 1-8, 1996

29 Kanawaza M, Yoshihara K, Abe H, Iwadate M, Watanabe K, Suzuki S, Endoh Y, Takita K, Sekikawa K, Takenoshita S, Ogata T and Ohto H: Effects of PSK on T and dendritic cells differentiation in gastric or colorectal cancer patients. Anticancer Res *25(1B)*: 443-449, 2005.

30 O'Hara RJ, Greenman J, MacDonald AW, Gaskell KM, Topping KP, Duthie GS, Kerin MJ, Lee PW and Monson JR: Advanced colorectal cancer is associated with impaired interleukin 12 and enhances interleukin 10 production. Clin Cancer Res 4: 1943-1948, 1998.

31 Shibata M, Nezu T, Kanou H, Abe H Takekawa M and Fukuzawa M: Decreased production of interleukin-12 and type 2 immune responses are marked in cachectic patients with colorectal and gastric cancer. J Clin Gastroenterol *34(4)*: 416-420, 2002.

32 Higaki S, Akazawa A, Nakamura H, Takekawa M and Fukuzawa M: Metaplastic polyp of the colon develops in response to inflammation. J Gastroenterol Hepatol *14(7)*: 709-714, 1999. *Erratum* in: J Gastroenterol Hepatol *14(8)*: 832, 1999.

33 Ricchi P, Pignata S, Iaffaioli RV and Daniele B: Cyclo-oxygenase inhibition in colorectal adenomas and cancer. J Clin Gastroenterol *37(4)*: 281-287, 2003.

34 Bernardeau-Mozer M and Chaussade S: Cyclooxygenase 2 inhibitors and colorectal cancer. Bull Cancer *291(Suppl2)*: S89-98, 2004.

35 Dempke W, Rie C, Grothey A and Schmoll HJ: Cyclooxygenase-2:a novel target for cancer chemotherapy? J Cancer Res Clin Oncol *127* :411-417, 2001.

36 Kojima M, Morisaki T, Uchiyama A, Doi F, Mibu R, Katano M and Tanaka M: Association of enhanced cyclooxygenase-2 expression with possible local immunosuppression in human colorectal carcinomas. Ann Surg Oncol *8*: 458-465, 2001.

37 Takahashi K, Tanaka S, Furuta K and Ichikawa A: Histamine H(2) receptormediated modulation of local cytokine expression in a mouse experimental tumor model. Biochem Biophys Res Commun *297*: 1205-1210, 2002.

38 Packard KA and Khan MM: Effects of histamine on Th1/Th2 cytokine balance. Int Immunopharmacol *3*: 909-920, 2003.

39 Elenkov IJ, Webster E, Papanicolaou DA, Fleisher TA, Chrousos GP and Wilder RL: Histamine potently suppresses human IL-12 and stimulates IL-10 production *via* H2 receptors. J Immunol *161*: 2586-2593, 1998.

40 Tomita K, Izumi K and Okabe S: Roxatidine and cimetidine-induced angiogenesis inhibition suppresses growth of colon cancer implants in syngeneic mice. J Pharmacol Sci *93*: 321-330, 2003.

41 Ding YH, Fu S, Zamarin D and Bromberg J: Interleukin-10. *In*: Thompson AW and Lotze MT: The Cytokine Handbook. Elsevier Science, 603-625, 2003.

42 Groux H, Bigler M, de Vries JE and Roncarolo MG: Interleukin-10 induces a long-term antigen-specific anergic state in human $CD4^+$ T-cells. J Exp Med *184*: 19-29, 1996.

43 Asselin-Paturel C, Megherat S, Vergnon I, Echchakir H, Dorothée G, Blesson S, Gay F, Mami-Chouaib F and Chouaib S: Differential effect of high doses *versus* low doses of interleukin-12 on the adoptive transfer of human specific cytotoxic T lymphocyte in autologous lung tumors engrafted into severe combined immunodeficiency disease-non obese diabetic mice: relation with interleukin-10 induction. Cancer *91*: 113-122, 2001.

44 Matsuda M, Salazar F, Petersson M, Masucci G, Hansson J, Pisa P, Zhang QJ, Masucci MG and Kiessling R: Interleukin 10 pre-treatment protects targe T-cells from tumor and allo-specific cytotoxic T-cells and downregulates HLA class I expression. J Exp Med *180*: 2371-2376, 1994.

45 Uotila P: The role of cyclic AMP and oxygen intermediates in the inhibition of cellular immunity in cancer. Cancer Immunol Immunother *43(1)*: 1-9, 1996.

46 Masini E, Fabbroni V, Giannini L, Vannacci A, Messerini L, Perna F, Cortesini C and Cianchi F: Histamine and histidine decarboxylase up-regulation in colorectal cancer: correlation with tumor stage. Inflamm Res *54(Suppl1)*: S80-81, 2005.

47 Asadullah K, Sterry W and Volk HD: Interleukin-10 therapy-review of a new approach. Pharmacol Rev *55*: 241-269, 2003.

48 van der Burg SH, Menon AG, Redeker A, Franken KL, Drijfhout JW, Tollenaar RA, Hartgrink HH, van de Velde CJ, Kuppen PJ, Melief CJ and Offringa R: Magnitude and polarization of P53-specific T-helper immunity in connection to leukocyte infiltration of colorectal tumors. Int J Cancer *107(3)*: 425-433, 2003.

49 Burgdorf SK, Claesson MH, Nielsen HJ and Rosenberg J: Changes in cytokine and biomarker blood levels in patients with colorectal cancer during dendritic cell based vaccination. Acta Oncol *27*: 1-8, 2009.

50 Chang CC, Campoli M and Ferrone S: HLA class I defects in malignant lesions: What have we learned? Keio J Med *52*: 220-229, 2003.

51 Diederichsen AC, Hansen TP, Nielsen O, Fenger C, Jensenius JC, Christensen PB, Kristensen T and Zeuthen J: A comparison of flow cytometry and immunohistochemistry in human colorectal cancers. APMIS *106(5)*: 562-570, 1998.

52 Diederichsen AC, Stenholm AC, Kronborg O, Fenger C, Jensenius JC, Zeuthen J, Christensen PB and Kristensen T: Flow cytometric investigation of immune response-related surface molecules on human colorectal cancers. Int J Cancer *79(3)*: 283-287, 1998.

53 Cabrera T, Collado A, Fernandez MA, Ferron A, Sancho J, Ruiz-Cabello F and Garrido F: High frequency of altered HLA class I phenotypes in invasive colorectal carcinomas. Tissue Antigens *52(2)*: 114-123, 1998.

54 Maleno I, Cabrera CM, Cabrera T, Paco L, López-Nevot MA, Collado A, Ferrón A and Garrido F: Distribution of HLA class I altered phenotypes in colorectal carcinomas: high frequency of HLA haplotype loss associated with loss of heterozygosity in chromosome region 6p21. Immunogenetics *56*: 244-53, 2004.

55 Romero JM, Jimenez P, Cabrera T, Cózar JM, Pedrinaci S, Tallada M, Garrido F and Ruiz-Cabello F: Coordinated downregulation of the antigen presentation machinery and HLA class I/beta2-microglobulin complex is responsible for HLA-ABC loss in bladder cancer. Int J Cancer *113*: 605-610, 2005.

56 Hicklin DJ, Marincola FM and Ferrone S: HLA class I antigen downregulation in human cancers: T-cell immunotherapy revives an old story. Mol Med Today *5*: 178-186, 2002.

57 Menon AG, Morreau H, Tollenaar RA, Alphenaar E, Van Puijenbroek M, Putter H, Janssen-Van Rhijn CM, Van De Velde CJ, Fleuren GJ and Kuppen PJ: Down-regulation of HLA-A expression correlates with a better prognosis in colorectal cancer patients. Lab Invest *82*: 1725-1733, 2002.

58 Rosenberg SA: Cancer vaccines based on the identification of genes encoding cancer regression antigens. Immunol Today *18*: 175, 1997.

59 Boon T and Old LJ: Cancer tumor antigens. Curr Opin Immunol *9(5)*: 681-683, 1997.

60 Ossendorp F, Mengedé E, Camps M, Filius R and Melief CJ: Specific T helper cell requirement for optimal induction of cytotoxic T lymphocytes against major histocompatibility complex class II negative tumors. J Exp Med *187(5)*: 693-702, 1998.

61 Toes RE, Ossendorp F, Offringa R and Melief CJ: CD4 T-cells and their role in antitumor immune responses. J Exp Med *189(5)*: 753-756, 1999.

62 Pardoll DM and Topalian S: The role of CD4+ T-cell responses in antitumor immunity. Curr Opin Immunol *10(5)*: 588-94, 1998.

63 Della PM, Danova M, Rigolin GM, Brugnatelli S, Rovati B, Tronconi C, Fraulini C, Russo Rossi A, Riccardi A and Castoldi G: Dendritic cells and vascular endothelial growth factor in colorectal cancer: correlations with clinicobiological findings. Oncology *68*: 276-84, 2005.

64 Gabrilovich DI, Chen HL, Girgis KR, Cunningham HT, Meny GM, Nadaf S, Kavanaugh D and Carbone DP: Production of vascular endothelial growth factor by human tumours inhibits the functional maturation of dendritic cells. Nat Med *2(10)*: 1096-1103, 1996. *Erratum* in: Nat Med *2(11)*: 1267, 1996.

65 Gabrilovich DI, Ishida T, Nadaf S, Ohm JE and Carbone DP: Antibodies to vascular endothelial growth factor enhance the efficacy of cancer immunotherapy by improving endogenous dendritic cell function. Clin Cancer Res *5(10)*: 2963-2970, 1999.

66 Malmberg KJ: Effective immunotherapy against cancer: a question of overcoming immune suppression and immune escape? Cancer Immunol Immunother *53*: 879-892, 2004.

67 Li Q, Pan PY, Gu P, Xu D and Chen SH: Role of immature myeloid GR-1+ cells in the development of antitumour immunity. Cancer Res *64*: 1130-1139, 2004.

68 Huang A, Gilmour JW, Imami N, Amjadi P, Henderson DC and Allen-Mersh TG: Increased serum transforming growth factor-beta1 in human colorectal cancer correlates with reduced circulating dendritic cells and increased colonic Langerhans cell infiltration. Clin Exp Immunol *134*: 270-278, 2003.

69 Yang L and Carbone DP: Tumour-host immune interactions and dendritic cell dysfunction. Adv Cancer Res *92* :13-27, 2004.

70 Qian Z, Chen L, Fernald AA, Williams BO and Le Beau MM: A critical role for APC in hematopoietic stem and progenitor cell survival. J Exp Med *205(9)*: 2163-2175, 2008.

71 Mailliard Rb and Lotze MT: Dendritic cells prolong tumor-specific T-cell survival and function after interaction with tumor targets. Clin Cancer Res *7*: 980-988, 2001.

72 Kusume A, Sasahira T, Luo Y, Isobe M, Nakagawa N, Tatsumoto N, Fujii K, Ohmori H and Kuniyasu H: Suppression of dendritic cells by HMGB1 is associated with lymph node metastasis of human colon cancer. Pathobiology *76(4)*: 155-62, 2009.

73 Curiel TJ, COukos G, Zou L, Alvarez X, Cheng P, Mottram P, Evdemon-Hogan M, Conejo-Garcia JR, Zhang L, Burow M, Zhu Y, Wei S, Kryczek I, Daniel B, Gordon A, Myers L, Lackner A, Disis ML, Knutson KL, Chen L and Zou W: Specific recruitment of regulatory T-cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat Med *10(9)*: 942-949, 2004.

74 Maloy KJ and Powrie F: Regulatory T-cells in the control of immune pathology. Nat Immunol *2*: 816-822, 2001.

75 Dieckmann D, Plottner H, Dotterweich S and Schuler G: Activated CD4+ CD25+ T-cells suppress antigen-specific CD4+ and CD8+ T-cells but induce a suppressive phenotype only in CD4+ T-cells. Immunology *115*: 305-314, 2005.

76 Wolf AM, Wolf D, Steurer M, Gastl G, Gunsilus E and Grubeck-Loebenstein B: Increase of regulatory T-cells in the peripheral blood of cancer patients. Clin Cancer Res *9*: 606-912, 2003.

77 Misra N, Bayry J, Lacroix-Desmazes S, Kazatchkine MD and Kaveri SV: Cutting edge: human CD4+CD25+ T-cells restrain the maturation and antigen-presenting function of dendritic cells. J Immunol *172*: 4676-4680, 2004.

78 Nomura T and Sakaguchi S: Naturally arising CD25+CD4+ regulatory T-cells in tumour immunity. Curr Top Microbiol Immunol *293*: 287-302, 2005.

79 Correale P, Cusi MG, Tsang KY, Del Vecchio MT, Marsili S, Placa ML, Intrivici C, Aquino A, Micheli L, Nencini C, Ferrari F, Giorgi G, Bonmassar E and Francini G: Chemo-immunotherapy of metastatic colorectal carcinoma with gemcitabine plus FOLFOX 4 followed by subcutaneous granulocyte macrophage colonystimulating factor and interleukin-2 induces strong immunologic and antitumor activity in metastatic colon cancer patients. J Clin Oncol *23*: 8950-8958, 2005.

80 Morse MA, Hobeika AC, Osada T, Serra D, Niedzwiecki D, Lyerly HK and Clay TM: Depletion of human regulatory T-cells specifically enhances antigen-specific immune responses to cancer vaccines. Blood *112(3)*: 610-618, 2008.

81 Matsuda M, Petersson M, Lenkei R, Taupin JL, Magnusson I, Mellstedt H, Anderson P and Kiessling R: Alterations in the signal-transducing molecules of T-cells and NK cells in colorectal tumor-infiltrating, gut mucosal and peripheral lymphocytes: correlation with the stage of the disease. Int J Cancer *61(6)*: 765-772, 1995.

82 Yoong KF and Adams DH: Interleukin 2 restores CD3-zeta chain expression but fails to generate tumour-specific lytic activity in tumour-infiltrating lymphocytes derived from human colorectal hepatic metastases. Br J Cancer *77*: 1072-1081.

83 De Paola F, Ridolfi R, Riccobon A, Flamini E, Barzanti F, Granato AM, Mordenti GL, Medri L, Vitali P and Amadori D: Restored T-cell activation mechanisms in human tumour-infiltrating lymphocytes from melanomas and colorectal carcinomas after exposure to interleukin-2. Br J Cancer *88*: 320-326, 2003.

84 Hoover HC Jr., Brandhorst JS, Peters LC, Surdyke MG, Takeshita Y, Madariaga J, Muenz LR and Hanna MG Jr.: Adjuvant active specific immunotherapy for human colorectal cancer: 6.5-year median follow-up of a phase III prospectively randomized trial. J Clin Oncol *11*: 390-399, 1993.

85 Fournier P and Schirrmacher V: Randomized clinical studies of anti-tumour vaccination: state of the art in 2008. Exp Rev Vaccines. Epub 01/30/2009 (Medscape).

86 Mayordomo JI, Loftus DJ, Sakamoto H, De Cesare CM, Appasamy PM, Lotze MT, Storkus WJ, Appella E and DeLeo

AB: Therapy of murine tumors with p53 wild-type and mutant sequence peptide-based vaccines. J Exp Med *183*: 1357, 1996.
87 Ciernik IF, Berzofsky JA and Carbone DP: Induction of cytotoxic T lymphocytes and antitumour immunity with DNA vaccines expressing single T-cell epitopes. J Immunol *156*: 263, 196.
88 DeLeo AB: p53-based immunotherapy of cancer. Crit Rev Immunol *18*: 29, 1998.
89 Vierboom MP, Nijman HW, Offrina R, van der Woort EI, van Hall T, van den Broek L, Fleuren GJ, Kenemans P, Kast WM and Melief CJ: Tumor eradication by wild-type p53-specific cytotoxic T lymphocytes. J Exp Med *186*: 695, 1997.
90 Roth J, Dittmer D, Rea D, Tartaglia J, Paoletti E and Levine AJ: P53 as a target for cancer vaccines: recombinant canarypox vecors expressing p53 protect mice against lethal tumor cell challenge. Proc Natl Acad Sci *93*: 4781, 1996.
91 Theobald M, Biggs J, Dittmer D, Levine J and Sherman LA: Targetting p53 as a general tumor antigen. Proc Natl Acad Sci *92*: 11993, 1995.
92 Yanuck M, Carbone DP, Pendleton CD, Tsukui T, Winter SF, Minna JD and Berzofsky JA: A mutant p53 tumor-suppressor protein is a target for peptide-induced $CD8^+$ cytotoxic T-cells. Cancer Res *53*: 3257, 1993.
93 Rogel A, Popliker M, Webb C and Oren M: p53 cellular tumor antigen: analysis of mRNA levels in normal adult tissues, embryos and tumors. Mol Cell Biol *5*: 2851, 1985.
94 Theobald M, Biggs J, Hernandez J, Lustgarten J, Labadie C and Sherman LA: Tolerance to p53 by A2.1-restricted cytotoxic T lymphocytes. J Exp Med *185*: 833, 1997.
95 Fedoseyeva EV, Boisgerault F, Anosova NG, Wollish WS, Arlotta P, Jensen PE, Ono SJ and Benichou G: $CD4^+$ T-cell responses to self-and mutated p53 determinants during tumorigenesis in mice. J Immunol *164*: 5641-5651, 2000.
96 Campo E, Palacin A, Benasco C, Quesada E, Cardesa A: Human chorionic gonadotropin in colorectal carcinoma: an immunohistochemical study. Cancer *59(9)*: 1611-1616, 1987.
97 Yamaguchi A, IshdaT, Nishimura G, Kumaki T, Katoh M, Kosaka T, Yonemura Y and Miyazaki I: Human chorionic gonadotropin in colorectal cancer and its relationship to prognosis. Br J Cancer *60(3)*: 382-384, 1989.
98 Shousha S, Chappell R, Matthews J and Cooke T: Human chorionic gonadotrophin expressed in colorectal adenocarcinoma. Dis Colon Rectum *29(9)*: 558-560, 1986.
99 Maghine M, Valtorta A, Moretta A, Priora C and Preti P: Effects of chorionic gonadotropin (hCG) therapy on the immune system. Medicina (Firenze) *10(2)*: 148-149, 1990.
100 Marshall JL, Hawkins MJ, Tsang KY, Richmond E, Pedicano JE, Zhu MZ and Schlom J: Phase I study in cancer patients of a replication-defective avipox recombinant vaccine that expresses human carcinoembryonic antigen. J Clin Oncol *17(1)*: 332-337, 1999.
101 Tsang KY, Zhu M, Nieroda CA, Correale P, Zaremba S, Hamilton JM, Cole D, Lam C and Schlom J: Phenotypic stability of a cytotoxic T-cell line directed against an immunodominant epitope of human carcinoembryonic antigen. Clin Cancer Res *3*: 2439-2449, 1997.
102 Wang RF and Rosenberg SA: Human tumour antigens for cancer vaccine development. Immunol Rev *170*: 85-100, 1999.
103 Jones H, Roberts G, Hole N, McDicken IW and Stern P: Investigation of expression of 5T4 antigen in cervical cancer. Br J Cancer *61(1)*: 96-100, 1990.
104 Southall PJ, Boxer GM, Bagshawe KD, Hole N, Bromley M and Stern PL: Immunohistological distribution of 5T4 antigen in normal and malignant tissues. Br J Cancer *61(1)*: 89-95, 1990.
105 Starzynka T, Rahi V and Starn PL: 5T4 oncofetal antigen expression in ovarian carcinoma. Int J Gynecol Cancer *5(4)*: 269-274, 1995.
106 Starzynka T, Marsh PJ, Schofield PF, Roberts SA, Myers KA and Stern PL: Prognostic significance of 5T4 oncofetal antigen expression in colorectal carcinoma. Br J Cancer *69(5)*: 899-902, 1994.
107 Carsberg CJ, Myers KA, Evans GS, Allen TD and Stern PL: Metastasis-associated 5T4 oncofoetal antigen is concentrated at microvillus projections of the plasma membrane. J Cell Sci *108(Pt 8)*: 2905-2916, 1995.
108 Carsberg CJ,m Myers KA and Starn PL: Metastasis-associated 5T4 antigen disrupts cell-cell contacts and induces cellular motility in epithelial cells. Int J Cancer *68*: 84-92, 1996.
109 Spano JP, Lagorce C, Atlan D, Milano G, Domont J, Benamouzig R, Attar A, Benichou J, Martin A, Morere JF, Raphael M, Penault-Llorca F, Breau JL, Fagard R, Khayat D and Wind P: Impact of EGFR expression on colorectal cancer patient prognosis and survival. Ann Oncol *16*: 102-108, 2005.
110 Goldstein NS and Armin M: Epidermal growth factor receptor immunohistochemical reactivity in patients with American Joint Committee on cancer stage IV colon adenocarcinoma. Cancer *92*: 1331-1346, 2001.
111 Yarden Y and Sliwkowski M: Untangling the ErbB signaling network. Nat Rev Mol Cell Biol *2*: 127-137, 2001.
112 Hung MC and Lau YK: Basic science of HER-2/neu: a review. Semin Oncol *26(4Suppl12)*: 51-59, 1999.
113 Schultz LB and Weber BL: Recent advances in breast cancer biology. Curr Opin Oncol *11(6)*: 429-434, 199.
114 Brossart P, Stuhler G, Flad T, Stevanovic S, Rammensee HG, Kanz L and Brugger W: Her-2/neu-derived peptides are tumor-associated antigens expressed by human renal cell and colon carcinoma lines and are recognized by *in vitro*-induced specific cytotoxic T lymphocytes. Cancer Res *58*: 732-736, 1998.
115 Agus DB, Bunn PA Jr, Franklin W, Garcia M and Ozols RF: HER-2/neu as a therapeutic target in non-small cell lung cancer, prostate cancer, and ovarian cancer. Semin Oncol *27(6 suppl11)*: 53-63; discussion 92-100, 2000.
116 Dufresne M, Seva C and Fourmy D: Cholecystokinin and gastrin receptors. Physiol Rev *86*: 805-847, 2006.
117 Ferrand A and Wang TC: Gastrin and cancer: a review. Cancer Lett *238*: 15-29, 2006
118 Bertrand C, Kowalski-Chauvel A, Do C, Résa C, Najib S, Daulhac L, Wang TC, Ferrand A and Seva C: A gastrin precursor, gastrin-gly, up-regulates VEGF expression in colonic epithelial cells through an HIF-1-independent mechanism. Int J Cancer *126(12)*: 2847-2857, 2010.
119 Guba M, Seeliger H, Kleespies A, Jauch KW and Bruns C: Vascular endothelial growth factor in colorectal cancer. Int J Colorectal Dis., 19: 510-517, 2004.
120 Vermorken JB, Claessen AM, van Tinteren H, Gall HE, Ezinga R, Meijer S, Scheper RJ, Meijer CJ, Bloemena E, Ransom JH, Hanna MG Jr. and Pinedo HM: Active specific immunotherapy for stage II and III colon cancer: a randomized trial. Lancet *353(9150)*: 345-350, 1999.

121 Harris JE, Ryan L, Hoover HC Jr., Stuart RK, Oken MM, Benson AB 3rd, Mansour E, Haller DG, Manola J and Hanna MG Jr: Adjuvant active specific immunotherapy for stage II and III colon cancer with an autologous tumor cell vaccine: Eastern Cooperative Oncology Group Study E5283. J Clin Oncol *18*: 148-157, 2000.

122 Uyl-de Groot CA, Vermorken JB, Hanna MG Jr., Verboom P, Groot MT, Bonsel GJ, Meijer CJ and Pinedo HM: Immunotherapy with autologous tumor cell-BCG vaccine in patients with colon cancer: a prospective study of medical and economic benefits. Vaccine *23*: 2379-2387, 2005.

123 Gray BN, Walker C, Andrewartha L, Freeman S and Bennett RC: Controlled clinical trial of adjuvant immunotherapy with BCG and neuraminidase-treated autologous tumour cells in large bowel cancer. J Surg Oncol *40(1)*: 34-37, 1989.

124 Gray BN, Walker C, Andrewartha L, Freeman S and Bennett RC: Melbourne trial of adjuvant immunotherapy in operable large bowel cancer. Aust N Z J Surg *58(1)*: 43-46, 1988.

125 Hanna MG Jr, Hoover HC Jr., Vermorken JB, Harris JE and Pinedo HM: Adjuvant active specific immunotherapy of stage II and stage II colon cancer with an autologous tumour cells vaccine: First randomised phase III trials show promise. Vaccine *19(17-19)*: 2576-2582, 2001.

126 Ockert D, Schirrmacher V, Beck N, Stoelben E, Ahlert T, Flechtenmacher J, Hagmüller E, Buchcik R, Nagel M and Saeger HD: Newcastle disease virus-infected intact autologous tumor cell vaccine for adjuvant active specific immunotherapy of resected colorectal carcinoma. Clin Cancer Res *(1)*: 21-28, 1996.

127 Schlag P, Manasterski M, Gerneth T, Hohenberger P, Dueck M, Herfarth C, Liebrich W and Schirrmacher V: Active specific immunotherapy with Newcastle disease virus-modified autologous tumor cells following resection of liver metastases in colorectal cancer. First evaluation of clinical response of a phase II-trial. Cancer Immunol Immunother *35(5)*: 325-330, 1992.

128 Schirrmacher V, Ahlert T, Pröbstle T, Steiner HH, Herold-Mende C, Gerhards R, Hagmüller E and Steiner HH: Immunization with virus-modified tumor cells. Semin Oncol *25(6)*: 677-696, 1998.

129 Schulze T, Kemmner W, Weitz J, Wernecke KD, Schirrmacher V and Schlag PM: Efficiency of adjuvant active specific immunization with Newcastle disease virus-modified tumour cells in colorectal cancer patients following resection of liver metastases: results of a prospective randomised trial. Cancer Immunol Immunother *58(1)*: 61-69, 2009.

130 Habal N, Gupta RK, Bilchik AJ, Yee R, Leopoldo Z, Ye W, Elashoff RM and Morton DL: CancerVax, an allogeneic tumor cell vaccine, induces specific humoral and cellular immune responses in advanced colon cancer. Ann Surg Oncol *8(5)*: 389-401, 2001.

131 Moulton HM, Yoshihara PH, Mason DH, Iversen PL and Triozzi PL: Active specific immunotherapy with β human chorionic gonadotrophin peptide vaccine in patients with metastatic colorectal cancer, antibody response is associated with improved survival. Clin Cancer Res *8(7)*: 2044-2051, 2002.

132 Phase I study of CDX-1307, β hCG vaccine, for patients with incurable, locally advanced or metastatic breast, colorectal, pancreatic, bladder or ovarian cancer. Celldex Therapeutics (NCT00648102).

133 Morse M, Chapman R, Powderly J, Keler T, He L, Ramakrishna V, Vitale L, Clay T, Green J and Davis T: Phase I clinical results of an APC-targeted hCGβ vaccine (CDX-1307) with TLR agonists. J Clin Oncol *27;15s (suppl;abstr 3006)*, 2009.

134 Lauwen MM, Zwaveling S, de Quartel L, Ferreira Mota SC, Grashorn JA, Melief CJ, van der Burg SH and Offringa R: Self-tolerance does not restrict the $CD4^+$ T-helper response against the p53 tumour antigen. Cancer Res *68(3)*: 893-900, 2008.

135 Speetjens FM, Kuppen PJ, Welters MJ, Essahsah F, Voet van den Brink AM, Lantrua MG, Valentijn AR, Oostendorp J, Fathers LM, Nijman HW, Drijfhout JW, van de Velde CJ, Melief CJ and van der Burg SH: Induction of p53-specific immunity by a p53 synthetic long peptide vaccine in patients treated for metastatic colorectal cancer. Clin Cancer Res *15(3)*: 1086-1095, 2009.

136 Kuball I, Schuler M, Antunes FE, Herr W, Neumann M, Obenauer-Kutner L, Westreich L, Huber C, Wölfel T and Theobald M: Generating p53-specific cytotoxic T lymphocytes by recombinant adenoviral vector-based vaccination in mice, but not man. Gene Ther *9*: 833-43, 2002.

137 Menon AG, Kuppen PJ, van der Burg SH, Offringa R, Bonnet MC, Harinck BI, Tollenaar RA, Redeker A, Putter H, Moingeon P, Morreau H, Melief CJ and van de Velde CJ: Safety of intravenous administration of a canarypox virus encoding the human wild-type p53 gene in colorectal cancer patients. Cancer Gene Ther *10*: 509-517, 2003.

138 van der Burg SH, Menon AG, Redeker A, Bonnet MC, Drijfhout JW, Tollenaar RA, van de Velde CJ, Moingeon P, Kuppen PJ, Offringa R and Melief CJ: Induction of p53-specific immune responses in colorectal cancer patients receiving a recombinant ALVAC-p53 candidate vaccine. Clin Cancer Res *8*: 1019-1027, 2002.

139 Antonia SJ, Mirza N, Fricke I, Chiappori A, Thompson P, Williams N, Bepler G, Simon G, Janssen W, Lee JH, Menander K, Chada S and Gabrilovich DI: Combination of p53 cancer vaccine with chemotherapy in patients with extensive stage small cell lung cancer. Clin Cancer Res *12(3 Pt 1)*: 878-887, 2006.

140 Svane IM, Pedersen AE, Johnsen HE, Nielsen D, Kamby C, Gaarsdal E, Nikolajsen K, Buus S and Claesson MH: Vaccination with p53-peptide-pulsed dendritic cells of patients with advanced breast cancer: report from a phase I study. Cancer Immunol Immunother *53*: 633-641, 2004.

141 Scartozzi M, Bearzi I, Berardi R, Mandolesi A, Fabris G and Cascinu S: Epidermal growth factor receptor (EGFR) status in primary colorectal tumors does not correlate with EGFR expression in related metastatic sites: implications for treatment with EGFR-targeted monoclonal antibodies. J Clin Oncol *22*: 4720-4726, 2004.

142 Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, Louis DN, Christiani DC, Settleman J and Haber DA: Specific activating mutations in the epidermal growth factor receptor and responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med *350*: 2129-2139, 2004.

143 Riemer AB, Kurz H, Klinger M, Scheiner O, Zielinski CC and Jensen-Jarolim E: Vaccination with cetuximab mimotopes and biological properties of induced anti-epidermal growth factor receptor antibodies. J Natl Cancer Inst *97(22)*: 1663-1670, 2005.

144 Kaumaya PT, Foy KC, Garrett J, Rawale SV, Vicari D, Thurmond JM, Lamb T, Mani A, Kane Y, Balint CR, Chalupa D, Otterson GA, Shapiro CL, Fowler JM, Grever MR, Bekaii-Saab TS and Carson WE 3rd: Phase I active immunotherapy with combination of two chimeric, human epidermal growth factor receptor 2, B-cell epitopes fused to a promiscuous T-cell epitope in patients with metastatic and/or recurrent solid tumors. J Clin Oncol *27(31)*: 5270-5277, 2009.

145 Gilliam AD and Watson SA: G17DT: an antigastrin immunogen for the treatment of gastrointestinal malignancy. Expert Opin Biol Ther *7(3)*: 397-404, 2007.

146 Watson SA and Gilliam AD: G17DT-A new weapon in the therapeutic armoury for gastrointestinal malignancy. Expert Opin Biol Ther *1(2)*: 309-317, 2001.

147 Rocha Lima CM, Buck R, Meyer K, Mitrica I, Perkins W and Michaeli DL: A multicenter phase II study of irinotecan in combination with G17DT immunogen in subjects with metastatic colorectal adenocarcinoma (CRC) refractory to previous irinotecan-based chemotherapy. J Clin Oncol, 2004 ASCO Ann Meet Proc *22(14S)*: 3573, 2004.

148 He AR and Marshall JL: Clinical experiences with G17DT in gastrointestinal malignancies. Exp Rev Anticancer Ther *6(4)*: 487-492, 2006.

149 Hörig H, Lee DS, Conkright W, Divito J, Hasson H, LaMare M, Rivera A, Park D, Tine J, Guito K, Tsang KW, Schlom J and Kaufman HL: Phase I clinical trial of a recombinant canarypoxvirus (ALVAC) vaccine expressing human carcinoembryonic antigen and the B7.1 co-stimulatory molecule. Cancer Immunol Immunother *49(9)*: 504-514, 2000.

150 Chen L: Co-inhibitory molecules of the B7-CD28 family in the control of T-cell immunity. Nat Rev Immunol *4*: 336-347, 2004.

151 Kaufman HL, Lenz HJ, Marshall J, Singh D, Garett C, Cripps C, Moore M, von Mehren M, Dalfen R, Heim WJ, Conry RM, Urba WJ, Benson AB3rd, Yu M, Caterini J, Kim-Schulze S, Debenedette M, Salha D, Vogel T, Elias I and Berinstein NL: Combination Chemotherapy and ALVAC-CEA/B7.1 vaccine in patients with metastatic colorectal cancer. Clin Cancer Res *14(15)*: 4843-4849, 2008.

152 Crossin KL, Chuong CM and Edelman GM: Expression sequences of cell adhesion molecules. Proc Natl Acad Sci USA *82(20)*: 6942-6946, 1985.

153 Edelman GM and Crossin K: Cell adhesion molecules: implication for a molecular histology. Annu Rev Biochem *60*: 155-190, 1991.

154 Litvinov SV, Bakker HA, Gourevitch MM, Velders MP and Warnaar SO: Evidence for a role of the epithelial glycoprotein 40 (Ep-CAM) in epithelial cell-cell adhesion. Cell Adhes Commun *2(5)*: 417-428, 1994.

155 Went P, Vasei M, Bubendorf L, Terracciano L, Tornillo L, Riede U, Kononen J, Simon R, Sauter G and Baeuerle PA: Frequent high-level expression of the immunotherapeutic target Ep-CAM in colon, stomach, prostate and lung cancers. Br J Cancer *94(1)*: 128-135, 2006.

156 Ullenhag GJ, Frödin JE, Mosolits S, Kiaii S, Hassan M, Bonnet MC, Moingeon P, Mellstedt H and Rabbani H: Immunization of colorectal carcinoma patients with a recombinant canarypox virus expressing the tumor antigen Ep-CAM/KSA (ALVAC-KSA) and granulocyte macrophage colony-stimulating factor induced a tumor-specific cellular immune response. Clin Cancer Res *9(7)*: 2447-2456, 2003.

157 Morse MA, Clay TM, Hobeika AC, Osada T, Khan S, Chui S, Niedzwiecki D, Panicali D, Schlom J and Lyerly HK: Phase I study of immunization with dendritic cells modified with fowlpox encoding carcinoembryonic antigen and costimulatory molecules. Clin Cancer Res *11(8)*: 3017-3024, 2005.

158 Marshall JL, Gulley JL, Arlen PM, Beetham PK, Tsang KY, Slack R, Hodge JW, Doren S, Grosenbach DW, Hwang J, Fox E, Odogwu L, Park S, Panicali D and Schlom J: 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 *23(4)*: 720-731, 2005.

159 Madan RA, Arlen PM and Gulley JL: PANVAC-VF: poxviral-based vaccine therapy targeting CEA and MUC1in carcinoma. Expert Opin Biol Ther *7(4)*: 543-554, 2007.

160 Gulley JL, Arlen PM, Tsang KY, Yokokawa J, Palena C, Poole DJ, Remondo C, Cereda V, Jones JL, Pazdur MP, Higgins JP, Hodge JW, Steinberg SM, Kotz H, Dahut WL and Schlom J: Pilot study of vaccination with recombinant CEA-MUC-1-TRICOM poxviral-based vaccines in patients with metastatic carcinoma. Clin Cancer Res *14(10)*: 3060-3069, 2008.

161 Harrop R, Ryan MG, Myers KA, Redchenko I, Kingsman SM and Carroll MW: Active treatment of murine tumours with a highly attenuated vaccinia virus expressing the tumour associated antigen 5T4 (TroVax) is CD4+ T-cell dependent and antibody mediated. Cancer Immunol Immunother *55(9)*: 1081-1090, 2006.

162 Abrams SI, Hodge JW, McLaughlin JP, Steinberg SM, Kantor JA and Schlom J: Adoptive immunotherapy as an *in vivo* model to explore antitumor mechanisms by a recombinant anticancer vaccine. J Immunother *20(1)*: 48-59, 2997.

163 Multyan K, Ryan MG, Myers KA, Shaw D, Wang W, Kingsman SM, Stern PL and Carroll MW: Attenuated recombinant vaccinia virus expressing oncofetal antigen (tumour-associated antigen) 5T4 induces active therapy of established tumors. Mol Cancer Ther *1(12)*: 1129-1137, 2002.

164 Harrop R, Connolly N, Redchenkko I, Valle J, Saunders M, Ryan MG, Myers KA, Drury N, Kingsman SM, Hawkins RE and Carroll MW: Vaccination of colorectal cancer patients with modified vaccinia ankara delivering the tumour antigen 5T4 induces immune responses which correlate with disease control: a phase I/II trial. Clin Cancer Res *12(11 Pt 1)*: 3416-3424, 2006.

165 De Cerio AL, Zabalegui N, Rodriguez-Calvillo M, Inoges S and Bendandi M: Anti-idiotype antibodies in cancer treatment. Oncogene *26(25)*: 3594-3602, 2007.

166 Foon KA, John WJ, Chakraborty M, Das R, Teitelbaum A, Garrison J, Kashala O, Chatterjee SK and Bhattacharya-Chatterjee M: Clinical and immune responses in resected colon cancer patients treated with anti-idiotype monoclonal antibody vaccine that mimics the carcinoembryonic antigen. J Clin Oncol *17(9)*: 2889-5, 1999.

167 Krug LM, Grant SC, Miller VA, Ng KK and Kris MG: Strategies to eradicate minimal residual disease in small cell lung cancer: high-dose chemotherapy with autologous bone marrow transplantation, matrix metalloproteinase inhibitors, and BEC2 plus BCG vaccination. Semin Oncol *26(5 Suppl 15)*: 55-61, 1999.

168 Posner MC, Niedzwiecki D, Venook AP, Hollis DR, Kindler HL, Martin EW, Schilsky RL, Goldberg RM and Mayer RJ: A phase II multi-institutional trial of adjuvant active specific immunotherapy following curative resection of colorectal cancer hepatic metastases: cancer and leukemia group B study 89903. Ann Surg Oncol *15(1)*:158-164, 2008.

169 Blumenthal RD: Technology evaluation: Onyvax-105. Curr Opin Mol Ther *5(6)*: 668-672, 2003.

170 Mikesch JH, Buerger H, Simon R and Brandt B: Decay-accelerating factor (CD55): a versatile acting molecule in human malignancies. Biochim Biophys Acta *1766*: 42-52, 2006.

171 Spendlove I, Ramage JM, Bradley R, Harris C and Durrant LG: Complement decay accelerating factor (DAF)/CD55 in cancer. Cancer Immunol Immunother *55*: 987-995, 2006.

172 Yu J, Caragine T, Chen S, Morgan BP, Frey AB and Tomlinson S: Protection of human breast cancer cells from complement-mediated lysis by expression of heterologous CD59. Clin Exp Immunol *115*: 13-18, 1999.

173 Nasu J, Mizuno M, Uesu T, Takeuchi K, Inaba T, Ohya S, Kawada M, Shimo K, Okada H, Fujita T and Tsuji T: Cytokine-stimulated release of decay-accelerating factor (DAF; CD55) from HT-29 human intestinal epithelial cells. Clin Exp Immunol *113(3)*: 379-385, 1998.

174 Maxwell-Armstrong CA, Durrant LG, Robins RA, Galvin AM, Scholefield JH and Hardcastle JD: Increased activation of lymphocytes infiltrating primary colorectal cancers following immunisation with the anti-idiotypic monoclonal antibody 105AD7. Gut *45(4)*: 593-598, 1999.

175 Maxwell-Armstrong C: Studies using the anti-idiotypic monoclonal antibody 105AD7 in patients with primary and advanced colorectal cancer. Ann R Coll Surg Engl *84(5)*: 314-318, 2002.

176 Maxwell-Armstrong, Durant LG, Buckley TJ, Scholefield JH, Robins RA, Fielding K, Monson JR, Guillou P, Calvert H, Carmichael J and Hardcastle JD: Randomised double blind phase II survival study comparing immunization with the anti-idiotypic monoclonal antibody 105AD7 against placebo in advanced colorectal cancer. Br J Cancer *84(11)*: 1443-1446, 2001.

177 Ullenhag GJ, Spendiove I, Watson NF, Indar AA, Dube M, Robins RA, Maxwell-Armstrong C, Scholefield JH and Durrant LG: A neoadjuvant/adjuvant randomised trial of colorectal cancer patients vaccinated with an anti-idiotypic antibody 105AD7, mimicking CD55. Clin Cancer Res *12(24)*: 7389-7396, 2006.

178 Samonigg H, Wilders-Truschnig M, Kuss I, Plot R, Stöger H, Schmid M, Bauernhofer T, Tiran A, Pieber T, Havelec L and Loibner H: A double-blind randomised phase II trial comparing immunisation with anti-idiotype goat antibody vaccine SCV 106 *versus* unspecific goat antibodies in patients with metastatic colorectal cancer. J Immunother *22(6)*: 481-488, 1999.

179 Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, Pulendran B and Palucka K: Immunobiology of dendritic cells. Annu Rev Immunol *18*: 767-811, 2000.

180 Ridgway D: The first 1000 dendritic cell vaccines. Cancer Invest *21(6)*: 873-86, 2003.

181 Banchereau J and Palucka AK: Dendritic cells as therapeutic vaccines against cancer. Nat Rev Immunol *5*: 296-306, 2005.

182 Figdor CG, de Vries IJ, Lesterhuis WJ and Melief CJ: Dendritic cell immunotherapy: mapping the way. Nat Med *10*: 475-480, 2004.

183 Wu YG, Wu GZ, Wang L, Zhang YY, Li Z and Li DC: Tumor cell lysate-pulsed dendritic cells induce a T-cell response against colon cancer *in vitro* and *in vivo*. Med Oncol 2009

184 Tamir A, Basagila E, Kagahzian A, Jiao L, Jensen S, Nicholls J, Tate P, Stamp G, Farzaneh F, Harrison P, Stauss H, George AJ, Habib N, Lechler RI and Lombardi G: Induction of tumor-specific T-cell responses by vaccination with tumor lysate-loaded dendritic cells in colorectal cancer patients with carcinoembryonic-antigen positive tumors. Cancer Immunol Immunother *56(12)*: 2003-2016, 2007.

185 Burgdorf SK, Fischer A, Myschetzky PS, Munksgaard SB, Zocca MB, Claesson MH and Rosenberg J: Clinical responses in patients with advanced colorectal cancer to a dendritic cell-based vaccine. Oncol Rep *20(6)*: 1305-1311, 2008.

186 Morse MA, Deng Y, Coleman D, Hull S, Kitrell-Fisher E, Nair S, Schlom J, Ryback ME and 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 *5*: 1331-1338, 1999.

187 Morse MA, Clay TM, Hobeika AC, Osada T, Khan S, Chui S, Niedzwiecki D, Panicali D, Schlom J and Lyerly HK: Phase I study of immunization with dendritic cells modified with fowlpox encoding carcinoembryonic antigen and co-stimulatory molecules. Clin Cancer Res *11*: 3017-3024, 2005.

188 Liu KJ, Wang CC, Chen LT, Cheng AL, Lin DT, Wu YC, Yu WL, Hung YM, Yang HY, Juang SH and Whang-Peng J: Generation of carcinoembryonic antigen (CEA)-specific T-cell responses in HLA-A*0201 and HLA-A*2402 late-stage colorectal cancer patients after vaccination with dendritic cells loaded with CEA peptides. Clin Cancer Res *10(8)*: 2645-2651, 2004.

189 Fong L, Hou Y, Rivas A, Benike C, Yuen A, Fisher GA, Davis MM and Engleman EG: Altered peptide ligand vaccination with Flt3 ligand-expanded dendritic cells for tumor immunotherapy. Proc Natl Acad Sci USA *98(15)*: 8809-8814, 2001.

190 Babatz J, Röllig C, Löbel B, Folprecht G, Haack M, Günther H, Köhne CH, Ehninger G, Schmitz M and Bornhäuser M: Induction of cellular immune responses against carcinoembryonic antigen in patients with metastatic tumors after vaccination with altered peptide ligand-loaded dendritic cells. Cancer Immunol Immunother *55(3)*: 268-276, 2006.

191 Nair SK, Morse M, Boczkowski D, Cumming RI, Vasovic L, Gilboa E and Lyerly HK: Induction of tumor-specific cytotoxic T lymphocytes in cancer patients by autologous tumour RNA-transfected dendritic cells. Ann Surg *235*: 540-549, 2002.

192 Lesterhuis WJ, de Vries IJ, Schuurhuis DH, Boullart AC, Jacobs JF, de Boer AJ, Scharenborg NM, Brouwer HM, van de Rakt MW, Figdor CG, Ruers TJ, Adema GJ and Punt CJ: Vaccination of colorectal cancer patients with CEA-loaded dendritic cells: antigen-specific T-cell responses in DTH skin tests. Ann Oncol *17(6)*: 974-980, 2006.

193 Jonuleit H, Giesecke-Tuettenberg A, Tüting T, Thurner-Schuler B, Stuge TB, Paragnik L, Kandemir A, Lee PP, Schuler G, Knop J and Enk AH: A comparison of two types of dendritic cell as adjuvants for the induction of melanoma-specific T-cell responses in humans following intranodal injection. Int J Cancer *93*: 243-251, 2001.

194 de Vries IJ, Lesterhuis WJ, Scharenborg NM, Engelen LP, Ruiter DJ, Gerritsen MJ, Croockewit S, Britten CM, Torensma R, Adema GJ, Figdor CG and Punt CJ: Maturation of dendritic cells is a prerequisite for inducing immune responses in advanced melanoma patients. Clin Cancer Res *9*: 5091-5100, 2003.

195 Dhodapkar MV, Krasovsky J, Steinman RM and Bhardwaj N: Mature dendritic cells boost functionally superior $CD8^{(+)}$ T-cell in humans without foreign helper epitopes. J Clin Invest *105*: R9-R14, 2000.

196 Hasegawa H, Mori M, Haraguchi M, Ueo H, Sugimachi K and Akiyoshi T: Expression spectrum of melanoma antigen-encoding gene family members in colorectal carcinoma. Arch Pathol Lab Med *122*: 551-554, 1998.

197 Kavanagh B, Ko A, Venook A, Margolin K, Zeh H, Lotze M, Schillinger B, Liu W, Lu Y, Mitsky P, Schilling M, Bercovici N, Loudovaris M, Guillermo R, Lee SM, Bender J, Mills B and Fong L: Vaccination of metastatic colorectal cancer patients with matured dendritic cells loaded with multiple major histocompatibility complex class I peptides. J Immunother *30(7)*: 762-772, 2007.

198 Park DI, Kang MS, Oh SJ, Kim HJ, Cho YK, Sohn CI, Jeon WK, Kim BI, Han WK, Kim H, Ryu SH and Sepulveda AR: HER-2/neu overexpression is an independent prognostic factor in colorectal cancer. Int J Colorectal Dis *22(5)*: 491-497, 2007.

199 Nagorsen D and Thiel E:Clinical and immunologic responses to active specific cancer vaccines in human colorectal cancer. Clin Cancer Res *12(10)*: 3064-2069, 2006.

200 Sallusto F and Lanzavecchia A: Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and down-regulated by tumor necrosis factor alpha. J Exp Med *179*: 1109-1118, 1994.

201 Romani N, Gruner S, Brang D, Kämpgen E, Lenz A, Trockenbacher B, Konwalinka G, Fritsch PO, Steinman RM and Schuler G: Proliferating dendritic cell progenitors in human blood. J Exp Med *180*: 83-93, 1994.

202 Jonuleit H, Kühn U, Müller G, Steinbrink K, Paragnik L, Schmitt E, Knop J and Enk AH: Pro-inflammatory cytokines and prostaglandins induce maturation of potent immunostimulatory dendritic cells under fetal calf serum-free conditions. Eur J Immunol *27*: 3135-3142, 1997.

203 de Vries IJ, Eggert AA, Scharenborg NM Vissers JL, Lesterhuis WJ, Boerman OC, Punt CJ, Adema GJ and Figdor CG: Phenotypical and functional characterization of clinical grade dendritic cells. J Immunother *25*: 429-438, 2002.

204 Morse MA, Nair S, Fernandez-Casal M, Deng Y, St Peter M, Williams R, Hobeika A, Mosca P, Clay T, Cumming RI, Fisher E, Clavien P, Proia AD, Niedzwiecki D, Caron D and Lyerly HK: Preoperative mobilization of circulating dendritic cells by Flt3 ligand administration to patients with metastatic colon cancer. J Clin Oncol *18*: 3883-3893, 2000.

205 Grover A, Kim GJ, Lizée G, Tschoi M, Wang G, Wunderlich JR, Rosenberg SA, Hwang ST and Hwu P: Intralymphatic dendritic cell vaccination induces tumor antigen-specific, skin-homing T lymphocytes. Clin Cancer Res *12(19)*: 5801-5808, 2006.

206 Saltz LB, Cox JV, Blanke C, Rosen LS, Fehrenbacher L, Moore MJ, Maroun JA, Ackland SP, Locker PK, Pirotta N, Elfring GL and Miller LL: Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. Irinotecan Study Group. N Engl J Med *343(13)*: 905-914, 2000.

207 Ferraresi V, Giampaolo MA, Gabriele A, Mansueto G, Buccilli A, Giannarelli D, Ciccarese M and Gamucci T: Activity and toxicity of oxaliplatin and bolus fluorouracil plus leucovorin in pretreated colorectal cancer patients: a phase II study. J Exp Clin Cancer Res *24*: 187-196, 2005.

208 Prete SP, Turriziani M, Massara MC, De Rossi A, Correale P, De Vecchis L, Torino F, Bonmassar L and Aquino A: Combined effects of 5-fluorouracil, folinic acid and oxaliplatin on the expression of carcinoembryonic antigen in human colon cancer cells: pharmacological basis to develop an active antitumor immunochemotherapy. J Exp Clin Cancer Res *27*: 5, 2008.

209 Harrop R, Drury N, Shingler W, Chikoti P, Redchenko I, Carroll MW, Kingsman SM, Naylor S, Melcher A, Nicholls J, Wassan H, Habib N and Anthoney A: Vaccination of colorectal cancer patients with modified vaccinia Ankara encoding the tumour antigen 5T4 given alongside chemotherapy induces potent immune responses. Clin Cancer Res *13(15 Pt 1)*: 4487-4494, 2007.

210 Harrop R, Drury N, Shingler W, Chikoti P, Redchenko I, Carroll MW, Kingsman SM, Naylor S, Griffiths R, Steven N and Hawkins RE: Vaccination of colorectal cancer patients with TroVax given alongside chemotherapy (5-fluorouracil, leukovorin and irinotecan) is safe and induces potent immune responses. Cancer Immunol Immunother *57(7)*: 977-986, 2008.

211 McWilliams JA, Sullivan RT, Jordan KR, McMahan RH, Kemmler CB, McDuffie M and Slansky JE: Age-dependent tolerance to an endogenous tumour associated antigen. Vaccine.2 *6*: 1863-1873, 2008.

212 Hoffmann TK, Loftus DJ, Nakano K, Maeurer MJ, Chikamatsu K, Appella E, Whiteside TL and DeLeo AB: The ability of variant peptides to reverse the nonresponsiveness of T lymphocytes to the wild-type sequence p53(264-272) epitope. J Immunol *168(3)*: 1338-1347, 2002.

213 Hemmer B, Stefanova I, Vergelli M, Germain RN and Martin R: Relationships among TCR ligand potency, thresholds for effector function elicitation and the quality of early signalling events in human T-cells. J Immunol *160*: 5807-5814, 1998.

214 Trojan A, Witzens M, Schultze JL, Vonderheide RH, Harig S, Krackhardt AM, Stahel RA and Gribben JG: Generation of cytotoxic T lymphocytes against native and altered peptides of human leukocyte antigen-$A^{*}0201$ restricted epitopes from the human epithelial cell adhesion molecule. Cancer Res *61*: 4761-4765, 2001.

215 Lustgarten J, Dominguez AL and Cuadros C: The $CD8^{+}$ T-cell repertoire against Her-2/neu antigens in neu transgenic mice is of low avidity with antitumor activity. Eur J Immunol *34*: 752-761, 2004.

216 Jordan KR, McMahan RH, Kemmler CB, Kappler JW and Slansky JE: Peptide vaccines prevent tumor growth by activating T-cells that respond to native tumor antigens. Proc Natl Acad Sci USA *107(10)*: 4652-4657, 2010.

217 Baars A, Claessen AME, Wagstaff J, Giaccone G, Scheper RJ, Meijer S, Schakel MJ, Gall HE, Meijer CJ, Vermorken JB, Pinedo HM and van den Eertwegh AJ: A phase II study of active specific immunotherapy and 5-FU/Leucovorin as adjuvant therapy for stage III colon carcinoma. Br J Cancer *86(8)*: 1230-1234, 2002.

218 Chong JM and Speicher DW: Determination of disulfide bond assignments and *N*-glycosylation sites of the human gastrointestinal carcinoma antigen GA733-2 (CO17-1A, EGP, KS1-4, KSA and Ep-CAM). J Biol Chem *276(8)*: 5804-5813, 2001.

219 Maruyama H, Zaloudik J, Li W, Sperlagh M, Koido T, Somasundaram R, Scheck S, Prewett M and Herlyn D: Cancer vaccines: single-epitope anti-idiotype vaccine *versus* multiple-epitope antigen vaccine. Cancer Immunol Immunother *49(3)*: 123-132, 2000.

220 Herlyn D, Somasundaram R, Zaloudik J, Jacob L, Harris D, Kieny MP, Sears H and Mastrangelo M: Anti-idiotype and recombinant antigen in immunotherapy of colorectal cancer. Cell Biophys *24-25*: 143-153, 1994.

221 Kim-Schulze S, Kim HS, Wainstein A, Kim DW, Yang WC, Moroziewicz D, Mong PY, Bereta M, Taback B, Wang Q and Kaufman HL: Intrarectal vaccination with recombinant vaccinia virus expressing carcinoembronic antigen induces mucosal and systemic immunity and prevents progression of colorectal cancer. J Immunol *181(11)*: 8112-8119, 2008.

222 Seok YJ, Kim KI, Yoo KH, Hwang-Bo J, Lee HH, Shon DH, Ko KS, Kang HS, Lee KJ, Oh DB, Joung YH and Chung IS: Expression and immunogenicity of a recombinant chimeric protein of human colorectal cancer antigen GA733-2 and an Fc antibody fragment in stably transformed *Drosophila melanogaster* S2 cells. Appl Biochem Biotechnol 2010.

223 Takaku S, Terabe M, Ambrosino E, Peng J, Lonning S, McPherson JM and Berzofsky JA: Blockade of TGF-beta enhances tumor vaccine efficacy mediated by $CD8^{(+)}$ T-cells. Int J Cancer *126(7)*: 1666-1674, 2010.

224 Figueredo A, Coombes ME and Mukherjee S: Adjuvant therapy for completely resected stage II colon cancer. Cochrane Database Syst Rev *16(3)*: CD005390, 2008.

225 Elkord E, Dangoor A, Drury NL, Harrop R, Burt DJ, Drijfhout JW, Hamer C, Andrews D, Naylor S, Sherlock D, Hawkins RE and Stern PL: An MVA-based vaccine targeting the oncofetal antigen 5T4 in patients undergoing surgical resection of colorectal cancer liver metastases. J Immunother *31(9)*: 820-829, 2008.

226 Elkord E, Dangoor A, Burt DJ, Southgate TD, Daayana S, Harrop R, Drijfhout JW, Sherlock D, Hawkins RE and Stern PL: Immune evasion mechanisms in colorectal cancer liver metastasis patients vaccinated with TroVax (MVA-5T4). Cancer Immunol Immunother *(10)*: 1657-1667, 2009.

*Received 20 November 2009*
*Revised 1 June 2010*
*Accepted 8 June 2010*