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 chemoradiotherapy 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.

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...
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 (IFN-gamma, TNF-alpha) 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 humoral 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 cyclooxygenase 2 (COX-2) (33-36), histamine (37-40) and IL10 (17, 41-44) 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 immunosuppression, 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 immunosuppression 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 p561ck (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.

**BhCG.** In contrast to other oncofoetal antigens, beta human chorionic gonadotrophin (BhCG) is not produced by normal colorectal cells (96-99). BhCG 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 BhCG is appealing as it might result in humoral 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 poor 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

1. Autologous Tumour Cell-derived Vaccines (Table 1). 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 TAA.

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-
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 vivo: 607-628 (2010)

### Table I. Autologous tumour cell vaccines.

<table>
<thead>
<tr>
<th>Vaccine type</th>
<th>Trial</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autologous tumour cells &amp; BCG</td>
<td>Phase III RCT</td>
<td>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.</td>
<td>Hoover et al. (1993) (84)</td>
</tr>
<tr>
<td>Autologous tumour cells &amp; BCG</td>
<td>Phase III RCT</td>
<td>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. Largerr 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.</td>
<td>Vermorken et al. (1999) (120)</td>
</tr>
<tr>
<td>Autologous tumour cells &amp; BCG</td>
<td>Phase III RCT</td>
<td>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.</td>
<td>Harris et al. (2000) (121)</td>
</tr>
<tr>
<td>Autologous tumour cells &amp; BCG</td>
<td>Phase III RCT</td>
<td>OS 65% in ASI group. Significant benefits in stage II colon cancer.</td>
<td>Hanna et al. (2001) (125)</td>
</tr>
<tr>
<td>Autologous tumour cells &amp; BCG (OncoVAX)</td>
<td>Phase III RCT</td>
<td>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.</td>
<td>Uyl-de Groot CA (2005) (122)</td>
</tr>
<tr>
<td>Autologous tumour cells &amp; virus ATV-NDV</td>
<td>Phase III RCT</td>
<td>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</td>
<td>Schulze et al. (2009) (129)</td>
</tr>
<tr>
<td>Autologous tumour cells &amp; virus ATV-NDV</td>
<td>Phase II RCT</td>
<td>2-Year survival rate 97% compared to ATV/BCG (66.7%).</td>
<td>Ockert et al. (1996) (126)</td>
</tr>
<tr>
<td>Autologous tumour cells &amp; virus ATV-NDV</td>
<td>Phase II RCT</td>
<td>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.</td>
<td>Schlag et al. (1992) (127)</td>
</tr>
<tr>
<td>Autologous tumour cells &amp; BCG</td>
<td>Phase II RCT</td>
<td>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)</td>
<td>Baars et al. (2002) (217)</td>
</tr>
</tbody>
</table>
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 TAAs, 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, CPT37-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, CPT37-DT generated a humoral 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 inoperable 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 antigen-based 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 Onyxvax-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.

<table>
<thead>
<tr>
<th>Vaccine type</th>
<th>Trial</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTP37-DT Avicin with BCG</td>
<td>Phase II RCT</td>
<td>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 ($p=0.0002$).</td>
<td>Moulton (2002) (131)</td>
</tr>
<tr>
<td>G17DT Gastroimmune to chemotherapy</td>
<td>Phase II</td>
<td>N/A</td>
<td>Gilliam (2007) (145)</td>
</tr>
<tr>
<td>G17DT Peptide vaccine</td>
<td>Phase II Combination chemotherapy</td>
<td>N/A</td>
<td>Rocha Lima et al. (2004) (147)</td>
</tr>
<tr>
<td>CDX-1307</td>
<td>Phase I</td>
<td>Ongoing</td>
<td>Morse (2009) (133)</td>
</tr>
<tr>
<td>P53 Synthetic long peptide vaccine</td>
<td>Phase II</td>
<td>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.</td>
<td>Speetjens et al. (2009) (135)</td>
</tr>
<tr>
<td>P53 Recombinant canarypox/ALVAC</td>
<td>Phase II</td>
<td>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.</td>
<td>van der Burg et al. (2002) (138)</td>
</tr>
<tr>
<td>PanVacVF, Recombinant fowlpox &amp; Vaccinia CEA/MUC-1</td>
<td>Phase I</td>
<td>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.</td>
<td>Gulley (2008) (160)</td>
</tr>
<tr>
<td>ALVAC-CEA/B7.1 Combination chemotherapy</td>
<td>Phase II RCT</td>
<td>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.</td>
<td>Kaufman (2008) (151)</td>
</tr>
<tr>
<td>ALVAC-KSA Combination chemotherapy</td>
<td>Phase II RCT</td>
<td>ALVAC-KSA, in combination with low dose local administration of GM-CSF may induce a strong, IFN-gamma-specific T-cell response.</td>
<td>Ullenhag (2003) (156)</td>
</tr>
<tr>
<td>ALVAC-p53 Recombinant canarypox virus/Ep-CAM/KSA and GM-CSF</td>
<td>Phase I-II escalation RCT</td>
<td>Potent T-cell and IgG antibody responses against the vector component of the ALVAC vaccine were induced in the majority of the patients.</td>
<td>Van der Burg (2002) (138)</td>
</tr>
<tr>
<td>rF-CEA(6D)-TRICOM infect dendritic cells &amp; co-stimulatory molecule</td>
<td>Phase I RCT</td>
<td>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. CEA-specific T-cell responses were observed in the majority of patients tested.</td>
<td>Morse (2005) (187) Marshall (2005) (158)</td>
</tr>
<tr>
<td>rv-CEA(6D)-TRICOM vaccinia vector to infect dendritic cells &amp; co-stimulatory</td>
<td>Phase I RCT</td>
<td>5T3-Specific antibody response generated in 94% of 22 vaccinated. No correlation between enhanced survival and MVA specific responses. 41% showed disease stabilisation.</td>
<td>Harrop et al. (2006) (161)</td>
</tr>
</tbody>
</table>
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 \( in \ text{vitro} \) and \( in \ text{vivo} \) (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). Immuni-

Table III. Anti-idiotype vaccines.

<table>
<thead>
<tr>
<th>Vaccine type</th>
<th>Trial</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CeaVac</td>
<td>Phase II RCT</td>
<td>Vaccine as adjuvant to chemotherapy and vaccine alone. CeaVac consistently generated a potent anti-CEA humoral and cellular immune response in all patients entered into this trial. 5-FU regimens: no effect on immune response.</td>
<td>Foon (1999) (166)</td>
</tr>
<tr>
<td>CeaVac &amp; Polyab</td>
<td>Phase II prospective</td>
<td>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.</td>
<td>Posner (2008) (168)</td>
</tr>
<tr>
<td>105AD7 (OnyVac) Anti-idiotype</td>
<td>Phase II RCT</td>
<td>Immune responses to vaccination were induced in a majority of monitored patients. RF survival, OS: NR.</td>
<td>Ullenlag (2006) (177)</td>
</tr>
<tr>
<td>SVC106 Anti-idiotype</td>
<td>Phase II RCT</td>
<td>Vaccination of immunologically responding metastatic colorectal carcinoma patients with SCV 106 leads to slowed disease progression and tumour dissemination and significantly prolongs survival time.</td>
<td>Samonigg (1999) (178)</td>
</tr>
<tr>
<td>5T3 TroVax Anti-idiotype MVA</td>
<td>Phase I/II</td>
<td>5T4-Specific immune responses in 94% of the 22 participants. No correlation between enhanced patient survival and MVA-specific immune responses. Overall, 41% patients showed disease stabilisation.</td>
<td>Harrop (2006) (161, 164)</td>
</tr>
<tr>
<td>5t3 TroVax</td>
<td>Phase II Adjuvant to chemotherapy</td>
<td>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&lt;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.</td>
<td>Harrop (2007) (209)</td>
</tr>
</tbody>
</table>
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 matured 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 leukaapheresis, 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 ‘immunchemotherapy’ (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 immuno-therapies 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/KS1A/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


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


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).


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