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Development of Modified Vaccinia Ankara-5T4 as Specific Immunotherapy for Advanced Human Cancer

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Abstract

Background—The tumor-associated antigen 5T4 is expressed on a high percentage of human carcinomas and has limited expression in normal tissues. A recombinant pox virus vector expressing this antigen, modified vaccinia Ankara (MVA)-5T4, has been tested as a cancer vaccine.

Objective—Treatment with MVA-5T4 has been studied both as a single agent and in combination with standard chemo-, biologic-, or targeted-therapies in patients with advanced colorectal cancer, renal cell carcinoma (RCC), or hormone-refractory prostate cancer.

Methods—This review summarizes data from clinical studies with MVA-5T4 reported in published manuscripts, meeting abstracts, or posted on websites relevant to clinical trials or MVA-5T4.

Results/conclusion—Vaccination with MVA-5T4 is well tolerated and elicits 5T4-specific humoral and/or cellular responses in the majority of treated patients. Retrospective analyses of phase II studies have suggested a positive association between immune responses to 5T4 and favorable clinical outcomes. An ongoing phase III, double-blind, placebo-controlled trial seeks to confirm a positive association between vaccination with MVA-5T4 and survival in patients with advanced RCC.

Keywords

5T4 Oncofetal Antigen; Cancer Vaccine; Cytotoxic T Lymphocyte; Modified Vaccinia Ankara-5T4; TroVax®

1. Introduction

Observations of rare spontaneous regressions of renal cell carcinomas, melanomas, and other tumors [1], the positive association of T cell infiltration into colorectal tumors with disease outcome [2], and evidence for naturally occurring T cells recognizing tumor-associated antigens in tumor patients [3] have been taken as evidence that cellular immune responses may play an important role in modulating tumor progression. Pioneering clinical studies by Rosenberg and co-workers have demonstrated that autologous tumor infiltrating T lymphocytes (TIL) expanded *in vitro* can be transferred to melanoma patients treated with lymphodepleting chemo or radiation therapy and achieve objective tumor regression in approximately 50% of treated patients (reviewed in [4]). Clinical responses have been associated with the lytic potency of the transferred cells for autologous tumor, the engraftment and *in vivo* persistence of the transferred cells, and the polyclonal composition and potentially

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the inclusion of $CD4^+$ T cells within the transferred TIL population. Although such a labor intensive and personalized approach to cancer therapy may not be practical for commercial application, the observation of robust T cell mediated antitumor effects provides the theoretical basis for clinical development of therapeutic cancer vaccines designed to elicit a specific cellular immune response targeting tumor-associated antigens.

1.1 The oncofetal antigen 5T4

Biologic characteristics common to placental and neoplastic cells including tissue invasion and escape from immunologic surveillance first suggested that discovery of "oncofetal" antigens shared on trophoblast and neoplastic cells might provide insight into tumor biology as well as identify potential diagnostic or therapeutic targets [5]. The 5T4 tumor antigen is a 420 amino-acid cell-surface glycoprotein that was identified as the target of a murine monoclonal antibody (mAb) raised by immunization with solubilized human syncytiotrophoblast plasma membranes [5,6]. 5T4 was shown to be highly expressed on human trophoblast cells and a majority of human tumors representing a wide range of histologies [5,7,8]. Although 5T4 expression was absent on most normal tissues, low level expression was demonstrated on the basal layer of stratified squamous epithelium, glandular and ductal epithelium, as well as neuronal subsets in the retina and olfactory bulb of mice [5,7,9,10].

Transfection of 5T4 cDNA into epithelial target cells *in vitro* resulted in changes in cell morphology associated with decreased cell-cell contact and increased cell motility [11]; features that might be associated with tumor dissemination and metastasis. An increased frequency of expression of 5T4 has been associated with more advanced disease in human cervical, colorectal, ovarian and gastric cancers [12-18]. Taken together, these observations suggested a possible role for 5T4 in the metastatic process.

1.2 Preclinical studies of MVA-5T4 (TroVax®)

Modified vaccinia virus Ankara (MVA) represents an attractive vector for cancer vaccine development. Isolated as a nonreplicating vaccinia strain for use in smallpox vaccination, there is substantial clinical experience with MVA attesting to its safe use in humans. Pox viruses including MVA have proven efficient vectors for recombinant gene expression tolerating integration of large amounts of DNA, and capable of stimulating transgene-specific cellular and humoral immune responses without a requirement for additional immune adjuvants [19].

Recombinant MVA vectors expressing human 5T4 (including the TroVax® vector further tested in human clinical studies [20]) have been evaluated in preclinical murine models for their capacity to elicit a 5T4-specific immune response and produce anti-tumor effects [20, 21]. Vaccination of mice with MVA-5T4 induced a potent antibody response that remained detectable for at least 6 months after the second of two vaccinations. A robust vector-specific CTL response could also be detected in the splenocytes of vaccinated mice [21], however 5T4-specific cytotoxicity was not observed, although a weak T cell response potentially 5T4 reactive was measured by ELISPOT [20]. Therefore, the capacity of CTL specific for 5T4 to directly target 5T4-expressing model tumor targets was not established in these studies.

To model antigen-specific anti-tumor therapy, mice were challenged by intravenous (i.v.) or subcutaneous (s.c.) inoculation with syngeneic colorectal or melanoma tumor cells transfected with human 5T4. MVA-5T4 vaccination at 3 and 10 days following tumor inoculation substantially reduced the number of pulmonary tumor nodules or delayed tumor outgrowth following i.v. or s.c. tumor challenge respectively [20,21]. Similar effects could be obtained with passive transfer of a monoclonal antibody or polyclonal serum specific for 5T4 suggesting much of the antitumor effect was mediated by the humoral immune response [20]. Thus, the

preclinical studies suggested the potential for MVA-5T4 vaccination to mediate anti-tumor effects in models of active treatment of established tumors.

2. Clinical Experience with MVA-5T4

MVA-5T4 was first tested as a single agent in patients with metastatic colorectal cancer (CRC) with responding or stable disease following first-line chemotherapy [22]. The primary objectives of this phase I/II study were to assess safety, biodistribution, and immunogenicity of the MVA-5T4 agent. Patient cohorts were treated with escalating doses of MVA-5T4 receiving 5×10^7, 2.5×10^8, or 5×10^8 plaque-forming units (p.f.u.) via intramuscular (i.m.) injection; or 1×10^8 p.f.u. via intradermal (i.d.) route. Twenty-two patients were enrolled and 17 completed three injections at 0, 4 and 8 weeks. Patients with a clinical or immunologic response were eligible to receive two additional vaccinations at 14 and 20 weeks (Table).

MVA-5T4 was well tolerated and no serious adverse events or autoimmune reactions related to MVA-5T4 administration were observed. Injection site soreness (i.m.) or erythema (i.d.) were the most common treatment-related adverse events. Consistent with the replication incompetent phenotype for the MVA vector, dissemination of MVA could not be detected in serial blood samples. Despite preceding treatment with chemotherapy, 5T4-specific antibody or proliferative responses were detected in 14/17 (82%) and 9/17 (53%) patients respectively with 16/17 (94%) of patients demonstrating a positive response for at least one of the immunologic assays. However, immune responses were often transient and detected at just a single post-vaccination timepoint for several patients (5/14 serologic responders and 5/9 proliferation assay responders) without clear correlations between vaccine dose level or route of administration and the titer or duration of the immune responses. The i.m. route of vaccination would be adopted for subsequent studies with MVA-5T4.

Notably, despite the presence of vector-specific neutralizing antibody in two patients prior to treatment with MVA-5T4, both patients developed both 5T4-specific serologic and proliferative responses. In addition, no correlation was noted between high titer of neutralizing antibody and weak 5T4-specific immune response [22]. Therefore, these observations supported a rationale for serial repeat vaccinations with MVA-5T4 with the intent to augment 5T4 specific immune responses.

A second study of MVA-5T4 as a single agent enrolled 20 CRC patients evaluated for resection of liver metastases [23]. Patients received two MVA-5T4 vaccinations prior to surgery and two vaccinations after surgery. Patients with positive serologic or cellular assays for a 5T4-specific immune response were offered two additional vaccinations at 20 and 28 weeks after surgery. The primary endpoint was immune response at the time of surgery. Of 20 enrolled patients, 16 completed the prescribed course of vaccinations and were eligible for immunologic assessment (Table). Similar to the initial phase I/II study of MVA-5T4, no serious adverse events were associated with MVA-5T4 administration. Assays for serologic or cellular immune responses to 5T4 were similarly positive in 14/16 (88%) and 12/16 (75%) of patients, respectively.

Given the initial success of MVA-5T4 administered as a single agent to elicit 5T4-specific humoral or cellular immune responses in the majority of treated CRC patients, MVA-5T4 has been further evaluated in a series of completed or ongoing phase II and phase III trials delivered in combination with standard of care chemo-, biologic-, or targeted-therapy regimens in patients with advanced CRC, renal cell carcinoma (RCC), or hormone-refractory prostate carcinoma (HRPC) (Table).

2.1 MVA-5T4 in metastatic CRC

MVA-5T4 was further evaluated in patients with metastatic CRC in two completed phase II trials of similar design undertaken to assess the safety and immunogenicity of MVA-5T4 vaccination given in combination with chemotherapy. MVA-5T4 was administered with first line chemotherapy comprised of 5-flurouracil (FU), leukovorin, and oxaliplatin [24] or 5-FU, leukovorin, and irinotecan [25]. A total of six MVA-5T4 vaccinations (5×10^8 pfu) were delivered in each study and included two before the start of chemotherapy, two during chemotherapy (maximum of twelve cycles), and two after completion of chemotherapy (Table). No serious adverse events related to MVA-5T4 vaccination were noted in either trial. A 5T4-specific antibody response was detected in 10/11 (91%) and 10/12 (83%) evaluable patients respectively. Peripheral blood mononuclear cells (PBMC) were also analyzed by ELISPOT assays for reactivity to a library of overlapping 10mer peptides spanning the entire 5T4 sequence [24,25]. The ELISPOT analysis represented a more direct and quantitative assay for cytotoxic T lymphocyte (CTL) reactivity to 5T4 than was previously measured by proliferative responses of PBMC to purified 5T4 protein [22]. A positive ELISPOT response to 5T4 peptides was detected in 9/11 (82%) and 10/12 (83%) of evaluable patients respectively in these two trials. In general, the magnitude and duration of 5T4-specific immune responses elicited by MVA-5T4 delivered in combination with front line chemotherapy in CRC patients was noted to be greater than that observed with MVA-5T4 as a single agent in the preceding phase I/II trial - perhaps reflecting a negative effect of prior chemo- or radiotherapy on the subsequent development of 5T4-specific immune responses.

While the initial trials of MVA-5T4 in CRC patients were not designed to assess clinical efficacy, retrospective analysis noted a statistically significant relationship between 5T4-specific antibody or ELISPOT responses and favorable clinical outcomes including time to progression ($P < 0.01$) [22] or RECIST response score ($P < 0.006$) and change in tumor burden ($P < 0.035$) [24], respectively, in two of the three published studies. Importantly, a similar relationship was not seen for vector-specific antibody or ELISPOT responses suggesting the potential association of 5T4-directed immune responses with anti-tumor effects.

2.2 MVA-5T4 in RCC

In a series of four phase II studies, MVA-5T4 treatment of patients with metastatic RCC has been tested in combination with IL-2 administered in high-dose or low-dose regimens or with IFN-α (Table). Preliminary findings regarding toxicity and immunogenicity for MVA-5T4 in this patient population were similar to studies conducted in CRC patients [26–29]. No serious adverse events were associated with administration of MVA-5T4 in RCC patients. Preliminary results from immunologic assays have been reported for three of these studies and indicated 5T4-specific immune responses were detectable in the majority of MVA-5T4 vaccinated RCC patients. All 22 patients treated with MVA-5T4 in combination with high-dose IL-2 developed detectable 5T4-specific humoral or cellular responses [29]. A 5T4-specific serologic response was detected in 21/25 (84%) patients who received MVA-5T4 in combination with low dose IL-2 [28]. Additionally, all 11 patients treated with MVA-5T4 in combination with IFN-α developed a 5T4-specific humoral response and 4/11 (36%) developed a 5T4-specific cellular response [27].

As seen with CRC patients, a subset of phase II studies of MVA-5T4 vaccination in RCC patients also observed a positive correlation between 5T4-specific immune response and positive clinical outcome. In patients treated with MVA-5T4 and low dose IL-2, a 5T4-specific humoral response was correlated with progression free and overall survival ($P < 0.05$ for both) [28]. In patients treated with MVA-5T4 and high-dose IL-2, patients with stable disease showed a significant increase in a 5T4-specific T cell response measured by ELISPOT assay compared to patients with progressive disease ($P = 0.005$) [29].

Based on the limited toxicity of MVA-5T4, favorable results from immune monitoring studies, and suggestion of the correlation of 5T4-specific immune response with favorable clinical outcomes seen in phase II studies, the TroVax Renal Immunotherapy Survival Trial (TRIST) was initiated in November 2006 as a phase III, randomized, placebo-controlled trial for locally advanced or metastatic clear cell RCC (Table). Treatment with MVA-5T4 vaccination in a front line setting was paired with standard of care systemic therapy that could include low-dose IL-2, IFN-α or the tyrosine kinase inhibitor sunitinib with patients receiving up to 13 vaccinations with MVA-5T4 (1×10^9 pfu) over 73 weeks. The primary study endpoint was survival [30]. Accrual of 733 patients was completed in March 2008 at more than 100 practice sites in the US, European Union and Eastern Europe. Following a predetermined fourth interim review by an independent Data Safety Monitoring Board (DSMB), the DSMB advised in July 2008 that the TRIST study will not meet the predefined primary efficacy endpoint noting a nonsignificant excess in the number of deaths on the TroVax® arm of the study and recommended that further vaccinations should be discontinued [31].

2.3 MVA-5T4 in HRPC

MVA-5T4 has also been investigated in two phase II studies in patients with HRPC (Table). Results from 27 patients treated with MVA-5T4 alone or in combination with granulocyte-macrophage colony stimulating factor (GM-CSF) identified no serious adverse events associated with MVA-5T4 in prostate cancer patients. Twenty-four patients were evaluable for immune responses demonstrating a 5T4-specific humoral or T cell response in 100% and 9/24 (38%) patients respectively. A significant association was noted between positive ELISPOT response and time to progression ($P = 0.024$) [32]. Results have not been reported from a second study with MVA-5T4 paired with docetaxel in HRPC patients [33].

3. Expert Opinion

The primary focus for most cancer vaccine therapies is their capacity to elicit a tumor-reactive T cell response. Despite the encouraging single-institution results with adoptive cellular therapy (ACT) for treatment of melanoma, currently no cancer vaccine designed to specifically stimulate tumor reactive T cells has been approved as a cancer therapy by the US FDA reflecting in general the failure of cancer vaccines to demonstrate potent anti-tumor effects *in vivo*. A recent review of 1,205 patients treated by cancer vaccines composed of peptides, recombinant virus, native or modified tumor cells, or dendritic cells that included 440 patients treated at the National Cancer Institute and 765 patients treated at other institutions noted overall objective response rates of just 2.6% and 3.8%, respectively [34]. Similarly, a meta-analysis of active specific immunotherapy for advanced colorectal cancer also noted an objective response rate of just 0.9% in 527 treated patients [35]. Such data have led to proposals for the investigation of cancer vaccines as a component of combination therapies [34] and the evaluation of patient benefit such as improved survival that might be observed despite low objective response rates measured by standard staging criteria [36].

The observation of 5T4 expression by most clear cell or papillary RCC tumors [8] and encouraging immunologic data from early phase human studies with the MVA-5T4 vaccine suggested the potential for additive or synergistic anti-tumor effects by combining MVA-5T4 with front line RCC-specific therapies (IL-2, IFN-α, or sunitinib). As a phase III trial, TRIST represents the most advanced clinical study of a specific immune therapy for RCC conducted to date. The conclusion by the DSMB following its fourth interim review that the TRIST trial will not meet its primary efficacy endpoint is therefore a disappointment; however, despite recommending the suspension of further vaccinations, the DSMB also advocated the continuation of the trial citing the potential for significant scientific merit to be gained [37]. Additional interim analysis reported by the sponsor suggested longer follow-up may be required to demonstrate a survival advantage for vaccinated patients [37]. The association of

a 5T4-specific immune response with favorable clinical outcome observed in early phase trials with MVA-5T4 was based on retrospective analyses allowing comparison of 5T4-responders to nonresponders. However, the fraction of patients mounting a 5T4-specific CTL response has varied widely among studies (range 36% – 83%). Therefore by intent-to-treat analysis, the potential for the TRIST study to measure a positive clinical effect for MVA-5T4 vaccination would be compromised by a high percent of 5T4 non-responders. In addition, the absence of tumor assessment for 5T4 expression in the entry criteria for studies with MVA-5T4 including the TRIST trial would allow for the inclusion of patients with tumors that are negative for the target antigen. Differential effects of the allowable combination therapies (IL-2, IFN-α, or sunitinib) on the evolution of an anti-tumor effector T cell population are also largely unknown.

A sustained and high level persistence of transferred tumor-specific CTL measured in the peripheral blood has been associated with clinical responses following ACT for melanoma [4] and may represent an important marker of a clinically effective cellular immune response. The quantitation of 5T4-specific CTL detected in peripheral blood following vaccination with MVA-5T4 has been reported primarily as patient-specific maximum CTL titers [24,25,32]. Thus, the optimal dose or schedule for repetitive MVA-5T4 administration to achieve maximal 5T4-specific CTL responses and sustain them over time has not been extensively investigated in early phase trials. Notably, interim analysis of TRIST trial data has suggested that less than 13 doses of MVA-5T4 may be necessary to optimize clinical efficacy [37]. Taken together, questions regarding the tempo of response, efficacy of treatment combinations, and optimal dosing schedule for MVA-5T4 as treatment for advanced RCC may yet be answered by mature TRIST trial data.

Further clinical development of MVA-5T4 in tumor histologies other than RCC is anticipated. Planning is ongoing for phase III trials with MVA-5T4 in CRC patients administered in the adjuvant setting or in combination with standard treatment of metastatic disease [38]. Additional insight into the potential for observing anti-tumor effects with MVA-5T4 would also benefit from correlative laboratory studies of the 5T4-specific CTL response elicited by this vaccine. *In vitro* functional data demonstrating the lytic potency of 5T4-specific CTL for RCC, colorectal, or other tumor targets have not been reported in either preclinical murine studies or in association with human clinical trials. The recent identification of CTL epitopes within 5T4 presented by common HLA-alleles such as HLA-A2 or HLA-Cw7 may facilitate such analyses [25,32,39,40].

Future development of MVA-5T4 might also take advantage of novel approaches to combination therapy. Development of effective ACT has been found to be enhanced by lymphodepletion prior to cell transfer possibly reflecting a reduction of regulatory T cells (Tregs) in the recipient [4]. Increased numbers of $CD4^+/CD25^+/FOXP3^+$ Tregs in peripheral blood and in tumor biopsies have been negatively correlated with clinical outcome for CRC and RCC [41,42] including RCC patients treated with MVA-5T4 vaccination plus high dose IL-2 [29]. Thus, the further investigation of MVA-5T4 vaccination in combination with strategies directed at depleting Tregs or at disrupting signals that may inhibit tumor-reactive cellular immune responses *in vivo* (such as mAb's targeting CTLA-4, PD-1, or PD-L1) represent a compelling direction for the ongoing clinical development of MVA-5T4 in metastatic RCC and other tumors.

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TABLE

Clinical Trials with MVA-5T4

Clinical Trials.gov Identifier	Trial Phase	Trial Format	Disease	MVA-5T4 Doses	Pfu/dose	Concomitant Systemic Treatment	Evaluable Patients	Study Status	Reference
Single Agent Studies									
None	I/II	Open-label	CRC	5	Dose escalation to 5 ×10^8	None	17	Completed	[22]
NCT00259844	II	Open-label	CRC	6	Not stated	None (surgery)	16	Completed	[23]
Combination Studies									
None	II	Open-label	CRC	6	5 × 10^8	5-FU/leukovorin/oxaliplatin	11	Completed	[24]
None	II	Open-label	CRC	6	5 × 10^8	5-FU/leukovorin/irinotecan	12	Completed	[25]
NCT00445523	II	Single center open-label	RCC	10	Not stated	None vs IFN-α	Not stated	Completed	[26]
None	I/II	Open-label	RCC	Not Stated	Not stated	IFN-α	11	Closed	[27]
NCT00325507	II	Single center open-label	RCC	8	Not stated	IL-2 (low dose)	25	Completed	[26,28]
NCT00083941	II	Single center open-label	RCC	8	Not stated	IL-2 (high dose)	22	Completed	[29]
NCT00397345	III	Multicenter randomized double-blind placebo-controlled	RCC	13	1 × 10^9	IFN-αIL-2 Sunitinib	733	Closed	[30]
NCT00448409	II	Single center open-label	HRPC	11	5 × 10^8	None vs GM-CSF	24	Completed	[32]
NCT00521274	II	Single center open-label	HRPC	12	Not stated	Docetaxel	60 (planned)	Stopped by Sponsor	[33]

Notes: abbreviations: pfu = plaque forming unit; CRC= colorectal cancer; RCC = renal cell carcinoma; HRPC = hormone-refractory prostate cancer; 5-FU = 5-fluoruracil; IFN-α = interferon-alfa; IL-2 = interleukin-2; GM-CSF = granulocyte-macrophage colony stimulating factor.