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Trovax, a recombinant modified vaccinia ankara virus encoding 5T4

Lessons learned and future development

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There has been renewed interest in developing vaccines and immunotherapy for the treatment of cancer. The oncofetal antigen, 5T4, is a surface glycoprotein that is expressed on a variety of human adenocarcinomas but rarely on normal tissue. 5T4 plays an important role in tumor progression and metastasis. The expression patterns and functional role in the metastatic process suggest that 5T4 is a good target for vaccine development. A modified vaccinia virus Ankara (MVA) encoding human 5T4 (designated TroVax) demonstrated therapeutic effects in murine tumor models and human T cells recognized 5T4 epitopes in an HLA-restricted manner. The TroVax vaccine has subsequently been evaluated in clinical trials targeting patients with colorectal cancer, renal cell carcinoma and hormone refractory prostate cancer. Herein, we review the results of these clinical studies, discuss the lessons learned through these trials and provide some insight into the future development of TroVax as a cancer vaccine.

Introduction

Cancer is a major public health threat accounting for 7.4 million or 13% of all deaths world wide in 2004.[1] According to the World Health Organization, the incidence of cancer will continue to increase over the next few decades with an expected 21.4 million new index cases annually and a doubling of the mortality to over 13 million annual deaths by 2030.[1] Thus, strategies for the treatment, prevention and control of cancer are of the highest priority. While initial enthusiasm for vaccines was dampened by a lack of therapeutic efficacy,[2] new data reported this year suggests that vaccines and other forms of immunotherapy, such as monoclonal antibodies that block CTLA-4, might have therapeutic benefit.[3-5] The reason for the improved efficacy of these new approaches might relate to improved selection of antigen(s), the potency of the vaccine/antibody platform, the disease histology tested, the design of the clinical trials and/or the selection of more appropriate clinical endpoints. In any event, the underlying mechanism of action for these agents is the induction of an adaptive immune response that is capable of recognizing one or more tumor-associated antigens that can mediate cytotoxic destruction of tumor cells. This response must be maintained as long as viable tumor cells remain in the host suggesting the need to generate a memory response, and this may be especially important in patients with established tumors. These principles are strongly supported by murine models of tumor immunotherapy but await confirmation in the clinical setting.

The identification of tumor-associated antigens is the foundation upon which modern tumor immunotherapy and vaccination is based. The optimal antigen remains to be defined but should be one that is unique to tumor cells, critical for tumor initiation and progression, recognized by the immune system and capable of mediating cytotoxicity of tumor cells.[6] Many defined tumor antigens possess some of these properties but few, if any, completely fulfill the criteria of tumor selectivity and potent immune response induction. The 5T4 glycoprotein is one antigen that is characterized by most of these criteria and is especially well suited for vaccine development.[7] The antigen was initially identified in a colorectal adenocarcinoma specimen but has since been found to be widely expressed on nearly all adenocarcinomas and rarely found on normal human tissues. The antigen was encoded in a recombinant modified vaccinia virus Ankara (MVA) vector for expression during vaccination. This vector was evaluated using the murine analog of 5T4 and demonstrated therapeutic effectiveness in a murine tumor model.[8,9] Additional studies using the human 5T4 identified HLA-A2-restricted T-cell epitopes within the coding sequence of the full-length 5T4 protein.[10] These pre-clinical studies provided the foundation for clinical development of the recombinant MVA-5T4 vector, designated TroVax. The vaccine has now been tested as a single agent, and in combination with immune adjuvants, in over 500 patients through a series of clinical trials in colorectal, prostate and renal cell carcinoma. This review will discuss the rationale for TroVax as a therapeutic cancer vaccine, detail the results of clinical studies completed to date, discuss the lessons learned from these studies and provide some insight into future directions for the clinical development of TroVax.

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Rationale for Development of TroVax as a Cancer Vaccine

1. Why is 5T4 a good tumor-associated antigen for vaccine development? 5T4 is a 72 kDa oncofetal glycoprotein that was discovered while searching for invasive molecules shared by tissue invasive placenta and tumor cells. The initial monoclonal antibody (H8) used to characterize 5T4 was isolated from a placental membrane extract and later 5T4 was found in a colorectal adenocarcinoma localized to the cell membrane but not shed into the circulation.[7,10] Further expression analysis studies have shown that 5T4 is expressed on trophoblast cells and most adenocarcinomas, including those of the kidney, bladder, breast, cervix, endometrium, lung, esophagus, ovary, pancreas, stomach, prostate and testicular non-seminomatous germ cell tumors.[11,12] The highest levels of 5T4 expression have been observed in carcinomas of the breast, gastrointestinal tract, ovaries and kidney (generally >70%), but rarely is 5T4 detected on normal tissues.[11,15] Further, 5T4 expression tends to increase with increasing tumor stage suggesting it might play a role in tumor progression. The only normal tissue with 5T4 expression is placenta and low levels have been identified on normal pituitary cells.

An important point is that 5T4 exists as a membrane-bound glycoprotein and is not released from the cell membrane. This has significant implications for tumor immunotherapy since surface 5T4 can mediate antibody-dependent cell-mediated cytotoxicity (ADCC), the lack of circulating soluble 5T4 might be associated with less immunosuppression and intra-cellular degradation of full-length 5T4 might be expected to generate T-cell specific epitopes. Furthermore, tumor expression of 5T4 has been associated with metastatic potential and poor prognosis in ovarian, colorectal and gastric cancer.[16-21] When tumor and epithelial cells are transfected with cDNA encoding 5T4, they display altered morphology and increased motility suggesting that 5T4 plays a role in tumor invasion and metastasis.[16,17] Recently, 5T4 precursor T cells were identified in healthy human subjects and both 5T4-specific $CD4^+$ and $CD8^+$ T-cell precursors could be expanded by stimulation with autologous dendritic cells infected with viral vector expressing 5T4.[10,23,24] In addition, 5T4 specific CD4 T cells were detected in a regressing renal cell carcinoma lesion.[23] Thus, the rather tumor selective expression of 5T4, cell membrane localization, lack of soluble protein, role in tumor progression and metastatic potential and presence of de novo precursor 5T4-specific T cells in healthy subjects all support the potential of 5T4 to serve as a potent tumor-associated antigen.

2. Why is MVA a good vector for 5T4 expression? Recombinant viral vectors have been widely studied as vectors for tumor antigen expression and the development of cancer vaccines. In particular, the poxviruses, including vaccinia virus, have been extensively investigated as recombinant vectors for vaccine development because they offer several noteworthy advantages over other viral vectors. First, the poxviruses are among the largest known mammalian viruses containing over 200 kB of viral DNA. This allows poxviruses to reliably replicate and express large eukaryotic transgenes or even multiple transgenes in a single vector.[25,26] Second, vaccinia virus vectors can generate potent cellular and humoral immune responses due to the inflammatory response triggered against highly immunogenic vaccinia proteins.[25] The induction of innate and adaptive immunity to the viral vector also seems to induce responses against foreign transgenes when expressed in recombinant poxvirus vectors.[27] Third, recombinant poxviruses have been able to break immune tolerance to "self" tumor antigens in several transgenic murine tumor models and in early phase clinical trials.[28-30] Fourth, recombinant poxviruses are generally easy to produce using in vitro cell culture and can be stored for long periods of time without losing potency. These advantages, however, are tempered by safety concerns with live, replicating vaccinia virus and the problem of limited boosting ability with these vectors due to the rapid appearance of strong anti-vaccinia neutralizing antibodies.[31]

Modified vaccinia virus Ankara (MVA) is highly attenuated vaccinia virus, which was initially developed as a safer vaccine during the smallpox eradication campaign in subjects at high risk for complications when exposed to vaccinia virus.[32] MVA was derived from multiple ex vivo passage of vaccinia virus and MVA has been shown to replicate only in a few selected human cell lines, making it safer than vaccinia virus. Recombinant MVA vectors have also demonstrated induction of immune responses in various infectious disease and tumor immunotherapy models with comparable or better levels of transgene-specific immunity.[33] In contrast to vaccinia virus, there were no complications reported when MVA was administered as a smallpox vaccine to over 120,000 human subjects, many of who were at risk for vaccinia vaccine complications.[32] Thus, MVA represents a logical choice for vaccine development and was used to express both murine and human 5T4 for the pre-clinical and clinical studies, respectively, as described below.

3. MVA-5T4 has therapeutic effectiveness in a murine tumor model. Preliminary studies of recombinant MVA encoding human 5T4 demonstrated both preventive and therapeutic effects in mice harboring colorectal cancer and melanoma tumors expressing human 5T4. These anti-tumor effects were associated with the induction of an anti-5T4 antibody response.[34,35] In a self-antigen melanoma model using the murine homologue of 5T4 (m5T4), MVA-m5T4 vaccination induced m5T4-specific antibody responses without signs of autoimmune toxicity.[34] In this model, vaccination prevented the establishment of syngeneic tumor cells expressing m5T4 for up to six months following vaccination.[35] The anti-tumor effect of MVA-h5T4 vaccine in mice appeared to be antibody related since passive transfer of 5T4 antibodies induced similar anti-tumor effects. 5T4-specific cytotoxic T-cell responses were not detected by IFNγ ELISPOT assay and $CD8^+$ T-cell depletion studies did not have any negative effect on tumor protection.[35] Overall, these studies have supported the rationale of MVA-5T4 vaccination in inducing therapeutic anti-tumor responses and provided rationale for further clinical development.

4. Identification of human HLA-restricted 5T4 epitopes. The main goal for therapeutic cancer vaccines is the induction of tumor-specific $CD8^+$ cytotoxic T lymphocytes (CTL), which can lyse tumor cells directly and generate long-term memory responses. In recent studies using ex vivo T cells derived from HLA-$A2^+$ patients, an overlapping pool of MHC class I restricted

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peptides was used to determine T-cell recognition.[36] Several HLA-A2-restricted peptides were found and could be used to expand CTL in vitro. These simple studies documented the ability of human T cells to recognize highly restricted 5T4 epitopes, supports the concept that vaccination can drive the expansion of such cytotoxic T cells and provides direct justification for the use of TroVax in the clinical setting.

Results of TroVax Clinical Trials

1. Colon cancer. The first clinical trial with TroVax was performed as a dose escalation Phase I trial in patients with advanced colorectal cancer who had responded or stabilized on first-line chemotherapy.[37] Twenty-two patients with stable metastatic (stage IV) colorectal cancer were given TroVax at 5 x 10^7 (1X), 2.5 x 10^8 (5X), 5 x 10^8 (10X) plaque-forming units (pfu) per milliliter by intramuscular (IM) injection; or 1 x 10^8 pfu (2X) by intradermal (ID) injection at 0, 4 and 8 weeks. A maximum of two TroVax booster injections were given to patients who had exhibited an immune or clinical response after the initial course of three immunizations. TroVax was safe and well tolerated at all doses and administration routes and no deleterious autoimmune reactions were observed. The most frequent events related to TroVax were grade I inflammation at the injection site. TroVax induced 5T4-specific T-cell proliferation in 15 of 17 evaluable subjects (88%) and antibody responses in 14 of 17 subjects (82%); these responses did not correlate with the route of administration.[37] No objective responses were seen but periods of disease stabilization ranging from 3–18 months was observed in five patients, all of whom had significant increases in 5T4-specific antibody titers following vaccination.

Further exploration of the Phase I data suggested that there was not a significant dose escalation effect between the 1X, 5X and 10X doses when given by the IM route using conventional needle injection, although the higher dose (10X) was associated with earlier initiation of 5T4-specific T-cell proliferative responses. The ID route was tested in a separate cohort in this trial but did not enhance immune responses significantly compared with IM delivery. Thus, this trial established the 10X dose and the IM route as the preferred route for TroVax administration.

A single TroVax injection was able to induce MVA-specific cellular and humoral response, although 5T4-specific immune responses were only evident following two or three vaccinations and 5T4-specific T-cell responses appeared to be quite transient, suggesting more than one injection and additional booster immunizations are required to break tolerance against 5T4. The presence of anti-MVA neutralizing antibody titers did not inhibit development of 5T4 specific immune responses after TroVax vaccination. In this study, 5T4 antibody response was correlated with increased time to progression ($p < 0.01$) and enhanced patient survival ($p < 0.0001$).[37] These data suggested that induction of 5T4-specific antibody titers was particularly important and might correlate with therapeutic responses, and could be boosted by TroVax even in the presence of pre-existing anti-MVA neutralizing antibody titers.

In an independent single institution, open label Phase II clinical trial, TroVax (10X) vaccinations were administered to 20 colorectal cancer patients before (two vaccinations at -4 and -2 weeks) and after (two vaccinations at +4 and +8 weeks) planned surgical resection of liver metastases.[38] Seventeen of 19 patients for whom tumor was available showed evidence of 5T4 expression within the resected metastatic lesions. TroVax vaccination induced 5T4-specific humoral immune responses in 18 of 19 (95%) evaluable patients. In this trial, another 8 of 16 (50%) patients showed 5T4-specific IFNγ ELISPOT responses using peripheral blood mononuclear cells (PBMC) and 3 of 9 (33%) patients using tumor-infiltrating lymphocytes after TroVax vaccination. The magnitude of 5T4-specific immune responses and high $CD3^+$ T-cell infiltration of the colon cancer metastases was significantly associated with increased survival in this study ($p = 0.05$ and $p = 0.012$ respectively).[38] Fifteen patients underwent a complete surgical resection of all metastatic disease and received all four planned vaccinations. A retrospective analysis of these 15 patients showed a trend toward improved survival in those patients demonstrating a higher 5T4-specific antibody and T-cell proliferative response. In patients exhibiting all three features, increased 5T4-specific antibody titers, 5T4-specific T-cell responses and tumor-infiltrating $CD3^+$ T cells, a statistically significant correlation with survival was seen (log rank, $p = 0.047$).

Recent data has suggested that cytotoxic chemotherapy may kill tumor cells releasing antigen and potentiating immunotherapy.[39] This observation suggests that vaccines might be an important adjuvant to standard cytotoxic chemotherapy of solid tumors. To test the role of TroVax in combination with chemotherapy, two open label Phase II studies in metastatic colorectal cancer patients were performed.[40,41] TroVax was injected before, during and after treatment with a combination of 5-flurouracil (5-FU), leucovorin and oxaliplatin (FOLFOX) or 5-FU, leucovorin and irinotecan (FOLFIRI). Administration of TroVax in combination with chemotherapy was safe and well tolerated with no serious adverse events reported. In particular, there was no enhancement of expected chemotherapy-related toxicity with either chemotherapy regimen. Potent 5T4-specific cellular and/or humoral immune responses were induced in all evaluable patients in both studies. Of note, 5T4-specific IFNγ ELISPOT responses were detected in 9 of 11 (82%) evaluable patients and in 10 of 12 (83%) evaluable patients in each study.[40,41] In each study, 5T4-specific antibody responses were also detected in 10 of 11 (91%) and 10 of 12 (83%) evaluable patients, respectively.

Although these clinical trials were designed as feasibility studies and not to detect clinical responses, it is noteworthy that 6 of 11 patients in the TroVax/FOLFOX trial achieved an objective complete or partial response. In addition, 5T4-specific cellular responses as detected by ELISPOT assay correlated with clinical benefit including RECIST response score ($p = 0.006$) and change in tumor burden ($p = 0.035$).[40] In the TroVax/FOLFIRI trial, of the 19 intention-to-treat subjects one had a complete response, 6 has a partial response and 5 had stable disease. Eight patients in this trial presented with elevations of serum CEA and six demonstrated a decrease of 50% or more during chemotherapy and four remained stable for over one month following completion of

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chemotherapy.[41] These studies support the safety and potential utility of combining TroVax with chemotherapy, in general. They also highlight the ability to induce 5T4-specific antibody and T-cell responses even in the setting of cytotoxic chemotherapy administration and around major surgical resection.

2. Prostate cancer. TroVax was evaluated in an open label phase II clinical trial in hormone refractory prostate cancer (HRPC) patients.[42] TroVax (10X) was administered by IM injection into the deltoid muscle of the upper arm on days 2, 13, 30, 41 and 58 either alone or combined with GM-CSF administered at a dose of 250 $\mu g/m^2$ by subcutaneous injection for 14 days in every 28 day cycle, for a total of 12 month. Following the initial vaccination period, three additional TroVax booster doses were given every 28 days until week 21 and another three boosters were given every 56 days until week 45. Men with confirmed adenocarcinoma of the prostate that had progressed after androgen deprivation and taxane-based chemotherapy were eligible for participation in this trial. Patients were required to have measurable disease as defined by RECIST criteria or an elevated PSA as defined by consensus criteria.[43] Patients were followed for clinical response by standard imaging and serum PSA levels.

Twenty-seven patients were enrolled in the trial with 14 assigned to TroVax alone and 13 assigned to TroVax and GM-CSF. Two patients deteriorated quickly and did not complete the initial vaccinations and were not considered evaluable and one patient was found to have hepatitis C and this patient was not included in the immune analysis. There were no serious adverse events and the most common toxicity was irritation at the vaccine site. Of the 24 evaluable patients, all developed 5T4-specific humoral response (100%) and 9 of 24 (38%) patients developed 5T4-specific T cell responses as determined by IFNγ ELISPOT assay. Although there were no objective clinical responses observed in the study, retrospective analysis revealed a significant improvement in time to disease progression for those nine patients who developed 5T4-specific T-cell responses compared to those patients who did not develop a T-cell response (5.6 vs. 2.3 months, p = 0.024).[42] There was no relationship between time to progression and 5T4 antibody titers in this trial. There was also no impact of GM-CSF on either clinical or immunological outcome.

3. Renal cell carcinoma. The fact that approximately 90% of clear cell and papillary renal cell carcinomas (RCC) express 5T4 and the inherent sensitivity of RCC to immunotherapy provided the motivation to test TroVax in patients with metastatic RCC. TroVax has now been investigated in four open label Phase II studies and a recently completed international, multi-institutional, placebo-controlled, randomized Phase III clinical trial in patients with metastatic renal cell carcinoma.[44-48] In the Phase II setting TroVax was combined with other biological adjuvant therapies, including high- and low-dose IL-2 and IFNα.[44-47] Overall, there were few serious adverse events attributed to TroVax in these studies suggesting the vaccine is safe and well tolerated in patients with RCC.

The first reported clinical trial was a Phase II study that evaluated TroVax with low-dose IL-2 in 25 patients with metastatic clear cell or papillary RCC.[44] TroVax was administered at the 10X dose by IM injection given two weeks before the start of IL-2 and three weeks after the first injection. IL-2 was administered in 8 week cycles starting with 250,000 units/kg/day for 5 days in week 1 followed by 125,000 units/kg/day for 5 days in weeks 2–6 by subcutaneous injection. Patients were treated for a maximum of six cycles and then were eligible to receive additional TroVax injections every 3 months if they were stable or had responding disease. The endpoints of the study included immune response and clinical responses assessed by standard RECIST criteria every 8 weeks. Of the 25 patients treated on this trial, 21 (84%) developed 5T4-specific antibody responses. Of 11 patients evaluable for T cell response, 6 (54%) developed MVA-specific T-cell responses and 5 (45%) patients developed 5T4-specific T-cell responses as measured by IFNγ ELISPOT assay.[44]

In this trial, two patients had a complete response that lasted for over 24 months and one patient had a partial response that lasted over 12 months. An additional six patients were stable for 6 to >21 months. The median progression-free survival was >3.4 months (range, 1.5–24.8 months). The overall survival was >12.9 months (range, 1.9–24.8 months). Of the eight patients with some clinical benefit, seven had clear cell and one had papillary histology. There was a trend toward improved progression-free survival in those patients with the highest median 5T4-specific antibody titers (p = 0.04) on retrospective analysis.

In another open label Phase II trial, 25 patients with metastatic clear cell or papillary RCC were treated with TroVax (10X) by IM injection every three weeks. Patients also received high-dose IL-2 at 600,000 units/kg intravenously every 8 hours according to standard high-dose IL-2 administration guidelines starting with the second TroVax vaccination.[45] The IL-2 was initiated on the same day as the TroVax injection and continued for five consecutive days. All patients received at least two cycles of IL-2 but could receive another two cycles in the absence of disease progression. Patients without progression were also eligible for booster TroVax injections every 3 months for one year.

In this trial, there were no serious adverse events related to TroVax. All 25 patients developed 5T4-specific antibody titers and 13 of 23 evaluable (57%) patients showed 5T4-specific $CD8^+$ T-cell responses by IFNγ ELISPOT assay.[45] There were no objective clinical responders in this trial but three patients were rendered free of disease by surgical resection of the primary RCC in two cases or small volume metastatic disease in one case. Twelve additional patients (48%) had stable disease, which was associated with improved median overall survival compared to patients without stable disease (not reached vs. 28 months, p = 0.026). In retrospective analysis of the immune response and clinical data, there was a significant increase in 5T4-specific $CD8^+$ T-cell response (p = 0.012) in patients with stable disease compared to patients with progressive disease.[45] Patients with stable disease also showed a significant increase in $CD8^+CD107^+$ (p = 0.015) and $CD8^+perforin^+$ (p = 0.02) T cells and a significant decrease in regulatory T cells (Tregs) defined as $CD4^+CD25^+FoxP3^+$ T cells (p = 0.006) in peripheral blood of patients exhibiting clinical benefit.

The third Phase II trial of TroVax in patients with metastatic RCC treated 28 patients with TroVax alone (n = 13) or TroVax

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and interferonα (n = 15).[46] Patients received TroVax at the 10X dose by IM injection on weeks 1, 3, 6, 9, 17, 28, 33 and 41. The combination cohort also received interferon-α at a dose of 6 million units three days a week by subcutaneous injection during week 1 followed by 9 million units three days a week by subcutaneous injection through week 48. As in the previous Phase II trials there were no serious adverse events attributed to TroVax.[46] There were 23 intention-to-treat patients available for immune analysis (three patients withdrew early and two patients had no baseline samples for comparison). Of the 23 patients evaluated, 22 (96%) developed 5T4-specific antibody responses. There were 21 patients who had enough PBMC available for IFNγ ELISPOT analysis and 7 of the 21 (33%) showed evidence of 5T4-specific T-cell responses. The median progression-free survival and overall survival for the 23 intention-to-treat patients was 3.8 months (range, 1–11.5 months) and 12.1 months (range, 1–27 months), respectively. There was a slight trend suggesting higher antibody responses in the group receiving TroVax and interferonα, while there was a slightly higher 5T4-specific T-cell response seen in the group receiving TroVax alone. There did not appear to be any clinical benefit for the addition of interferonα to TroVax treatment.

A smaller Phase I/II trial enrolled 11 metastatic RCC patients to receive TroVax in combination with interferonα. The goal of this trial was to determine the safety of the combination and see if immune responses could be detected.[47] In addition, clinical responses were determined by RECIST criteria and were documented in an exploratory manner. Similar to the other Phase II studies, there were no serious adverse events attributed to TroVax. All patients developed 5T4-specific antibody responses and 5 of 11 (45%) patients mounted 5T4-specific T-cell responses. Although no objective tumor responses were seen, the median time to progression was 9 months (range, 2.1–26 months) and 10.4 months for the clear cell patients (range, 3.9–26 months).

These Phase II trials established the safety profile of TroVax in patients with metastatic RCC and suggested that the vaccine was able to induce 5T4-specific antibody titers in nearly all patients. The studies also highlighted the induction of 5T4-specific $CD8^+$ T cells in many patients and showed a trend toward improved survival in those patients demonstrating the highest magnitude of immune response. Although clinical responses were not uniformly observed, these studies were not designed to detect significant changes in clinical outcome. Collectively, these studies supported the further development of TroVax in RCC and culminated in an international, multi-institutional, placebo-controlled, randomized Phase III clinical trial.

4. Phase III trials of trovax. The TroVax Renal Immunotherapy Survival Trial (TRIST) was designed to determine if the addition of TroVax to currently available standard of care therapy could improve survival for patients with metastatic RCC. This was an international, multi-institutional trial that prospectively randomized patients to 13 injections of TroVax or placebo along with standard first line treatment.[48] The major endpoint of the trial was overall survival and progression-free survival, objective response rate and safety were also determined as secondary endpoints. A total of 733 patients with locally advanced or metastatic clear renal cell carcinoma were treated with physician's choice first line standard of care (low dose IL-2, IFNα or the tyrosine kinase inhibitor sunitinib) and randomized 1:1 to additional therapy with TroVax or placebo. Following the fourth scheduled Data Safety Monitoring Board (DSMB) review in July of 2008, the DSMB recommended that further vaccinations be discontinued since the pre-defined primary endpoint would not be met. The DSMB, however, did advocate the continuation of the blinded study without further vaccinations due to important scientific merit and data to be learned by further follow-up.

Of the 733 patients enrolled on the TRIST trial, 732 were included in the intention-to-treat population. There were 37 subjects enrolled from the US, 92 recruited from European Union countries and 604 were from eastern Europe. The patients were split so 365 patients were randomized to TroVax and 368 patients received placebo. The standard of care therapies included 191 patients who received interferonα (9–18 million units by subcutaneous injection three times a week), 83 who received low-dose IL-2 (initial dose of 250,000 units/kg (with an upper limit of 22 million units/dose) for 5 days in week 1 of each cycle followed by 125,000 units/kg (with an upper limit of 11 million units/dose) for 5 days in each of weeks 2–6 of each cycle) and 93 who received sunitinib (50 mg by mouth daily for 4 weeks and 2 weeks off for each 6 week cycle). Overall the vaccine was well tolerated with few serious adverse events related to vaccination. The most frequently reported adverse events were low-grade fever, fatigue nausea and weight loss. There was no difference in the rate of adverse events between the TroVax and placebo groups.

The survival data was censored in March of 2009 at a median follow-up of 12.9 months. The median overall survival was 20.1 and 19.2 months for TroVax and placebo-treated patients, respectively (HR = 1.07; 95% CI 0.86–1.32; p = 0.55). Although no survival benefit was seen for the population as a whole, subset analysis revealed patients with a favorable prognostic index (MSKCC grade) and treated with TroVax and IL-2 showed a significant survival advantage compared to patients receiving placebo and IL-2 (HR = 0.54; 95% CI 0.30–0.98; p = 0.046). 5T4-specific antibody responses were detected in 60% of patients treated with TroVax and the magnitude of the 5T4-specific, but not the MVA-specific antibody response, was associated with improved survival (p = 0.04),[49] Exploratory analysis of the TRIST trial have also shown that low baseline platelet levels were associated with higher 5T4-specific antibody titers and improved survival (p = 0.04).[49]

Conclusions and Future Directions

The TroVax vaccine has now been tested in over 500 cancer patients through 10 clinical trials and has demonstrated a favorable toxicity profile without serious adverse events or detrimental autoimmunity. **Table 1** summarizes all of the clinical trials completed to date. Immune analysis in these trials has shown that TroVax generally induces 5T4-specific antibody titers in most patients and 5T4-specific T cell responses in a fewer number of patients. Retrospective analyses in many of the clinical studies demonstrated similar findings that suggest a correlation between the magnitude of the 5T4-specific immune response

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Table 1. A summary of all TroVax clinical trials completed prior to 2010

Disease	Phase	Sample size (N)	Concomitant treatment	Number of injections	Dose (pfu/ml)	Clinical outcome	Immunology outcome	Reference
Colorectal cancer	I/II	22	No	5	dose escalation to 5 x 10^8	CR:0/17 PR:0/17 SD:5/17 PD:12/17	5T4 antibody: 14/17 (82%) 5T4 protein specific proliferative response (PBMC): 11/17 5T4 peptide specific proliferative response (PBMC): 15/17	37
Colorectal cancer	II	20	No	6	5 x 10^8	Not stated	5T4 antibody: 18/19 5T4 specific proliferative response (PBMC): 13/20 5T4 specific IFNγ ELSPOT response (PBMC): 8/16 5T4 specific IFNγ ELSPOT response (TIL): 3/9	38
Colorectal cancer	II	17	5-FU/leukovorin/oxaliplatin	6	5 x 10^8	CR:1/11 PR:5/11 SD:1/11 PD:4/11	5T4 antibody: 10/11 5T4 specific IFNγ ELSPOT response (PBMC): 9/11	40
Colorectal cancer	II	19	5-FU/leukovorin/irinotecan	6	5 x 10^8	CR:1/12 PR:0/12 SD:0/12 PD:10/12	5T4 antibody: 10/12 5T4 specific IFNγ ELSPOT response (PBMC): 10/12	41
Renal cell carcinoma	II	25	Low dose IL-2	8	5 x 10^8	CR:2/25 PR:1/25 SD:6/25 PD:15/25	5T4 antibody: 21/25 5T4 specific IFNγ ELSPOT response (PBMC): 5/11	44
Renal cell carcinoma	II	25	High dose IL-2	8	5 x 10^8	CR:0/25 PR:0/23 SD:12/23 (including 3 surgical CR) PD:11/23	5T4 antibody: 23/23 5T4 specific IFNγ ELSPOT response (PBMC): 13/23	45
Renal cell carcinoma	I/II	11	IFNα	11	5 x 10^8	CR:0/11 PR:0/11 SD:10/11 PD:1/11	5T4 antibody: 11/11 5T4 specific IFNγ ELSPOT response (PBMC): 5/11	46
Renal cell carcinoma	II	28	None vs. IFNα	9	5 x 10^8	CR:0/25 PR:1/25 SD:14/25 PD:10/25	5T4 antibody: 21/25 5T4 specific IFNγ ELSPOT response (PBMC): 7/21	47
Renal cell carcinoma	III	733	Low dose IL-2, IFNα or sunitinib	13	1 x 10^9	Not stated	5T4 antibody: 60%	48
Hormone refractory prostate cancer	II	27	None vs. GM-CSF	11	5 x 10^8	CR:0/25 PR:0/25 SD:0/25 PD:25/25	5T4 antibody: 24/24 5T4 specific IFNγ ELSPOT response (PBMC): 9/24	42

CR, complete response; PD, progressive disease; PR, partial response.

and improved clinical outcomes. TroVax has been tested alone and in combination with a variety of other agents, including interferonα, GM-CSF, low- and high-dose IL-2 and sunitinib without significant changes in the safety profile of the vaccine. TroVax has also been tested in several different tumors, including colorectal cancer, prostate cancer and most extensively in renal cell carcinoma.

Although the Phase II studies suggested some clinical benefit, the Phase III trial in RCC was disappointing and failed to document a survival advantage for TroVax. This may relate, however, to several problems in the design of the Phase III TRIST trial. First, the optimal dosing, route of administration and boosting schedule have not been firmly established and could influence the potency of the immune response and possibly the therapeutic responses as well. Early phase TroVax trial data suggested that 5T4-specific immune responses required at least two or three vaccination and these responses, especially T-cell responses, were often transient.[37,38] Thus, it is

possible that a more accelerated vaccination schedule or ongoing booster immunizations will be needed to have a more significant impact on clinical outcomes. This may be an especially important point given the recent evidence of delayed kinetics in the clinical response to other forms of immunotherapy such as the anti-CTLA4 monoclonal antibody treatment.[50] The possibility that delayed clinical responses might be seen with TroVax are supported by the large number of stable disease patients reported in many of the Phase II clinical trials. Further, the design of the Phase III TRIST trial was complicated by the concurrent administration of TroVax with three other agents based on physician choice and the lack of data on how best to sequence TroVax with these other agents. There is also no data on how TroVax might interact with sunitinib. Thus, further investigation of the optimal dosing, route of administration and boosting schedule of TroVax might be helpful.

A second issue with TroVax related to the selection of appropriate patients for treatment with a vaccine. While there are currently no predictive biomarkers for patients likely to respond to immunotherapy, the clinical trials reported thus far, suggest that certain subgroups of patients may be more likely to achieve benefit with TroVax. Specifically, clinical benefits was more likely in patients exhibiting strong 5T4-specific immune responses and in good prognosis patients, as scored by MSKCC grading criteria, in patients treated with low dose IL-2 and TroVax.[48,49] Exploratory analyses in the TRIST Phase III study further identified baseline platelet counts as a predictor of 5T4-specific antibody response and clinical benefit. These criteria need further validation in prospective clinical trials but one could imagine that future eligibility criteria might include careful screening to exclude patients unlikely to respond to TroVax.

A third potential pitfall in the analysis of the TroVax trials is the issue of 5T4 expression. While expression analysis studies have indicated that 5T4 is expressed on over 70% of primary carcinomas of the breast, GI tract, ovaries and kidney, it is theoretically possible that some carcinomas may not express 5T4 or may exhibit very low levels of expression.[11-16] The loss of antigen expression has been documented as a major mechanism of immune escape used by established tumors. Further data on 5T4 expression patterns at the time of initial patient screening might be worthwhile to investigate in future trials.

A fourth potential obstacle to successful vaccination with TroVax is the presence of an immune suppressive microenvironment in patients with advanced cancers that might limit the effectiveness of the vaccine and might inadvertently induce regulatory and/or suppressor populations of cells that will block tumor eradication.[51] This possibility has been further suggested by data from early Phase TroVax clinical trials, in which loss of tumor HLA class I expression and increased expression of immune inhibitory factors, such as TGFβ, PD-1^{+} T cells and systemic and local regulatory T cells were reported.[52] Reversing the immune suppressive tumor microenvironment or depletion of local immune suppressive molecules or regulatory cells has been associated with enhanced anti-tumor immunity in animal models.[53,54] In fact, Treg depletion prior to 5T4 antigen stimulation in vitro was shown to enhance expansion of 5T4-specific T cells.[55] Thus, a potentially important area of future research will be studies that combine TroVax with inhibition of local tumor suppressor mechanisms.

In summary, TroVax is a novel vaccine based on an MVA vector encoding human 5T4, a commonly expressed cell surface tumor-associated antigen. Early phase clinical trials in patients with advanced colorectal, prostate and renal cell cancer have established the safety profile of this vaccine and demonstrated the induction of 5T4-specific humoral responses in most patients and T-cell responses in some patients. Although the Phase III clinical trial of TroVax in RCC failed to demonstrate an improvement in overall survival, the study did support the potential benefit of TroVax administered with IL-2 in subset analysis and also identified several clinical factors that might be predictive of clinical benefit. Future studies are needed to better define the dosing and schedule of vaccination in cancer patients, the optimal adjuvant and schedule for combination treatments, how to better select patients who might benefit from vaccination and how to inhibit tumor escape mechanisms that block 5T4-specific immunity in patients with cancer.

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