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TroVax®

Cancer Vaccine

MVA-5T4
SAR-109659

Modified vaccinia Ankara (MVA) virus strain encoding the antigen 5T4

EN: 317847

Abstract

Active specific immunization against cancer is a compelling concept which has been under investigatation for several decades. Among tumor vaccine approaches, a modified vaccinia Ankara (MVA) virus vector encoding the oncofetal antigen 5T4 (TroVax®) is one of the most advanced. Here, we summarize clinical and immunological data on TroVax® in various malignant diseases. The induction of a specific immune response was seen in the majority of patients and across all clinical trials, and preliminary clinical results, particularly in renal cell cancer (RCC), are encouraging. However, it is necessary to await results from larger ongoing trials to draw firm conclusions on the role of TroVax® in future oncological therapy.

Background

The mainstay of cancer treatment remains optimal surgical resection. With increasing frequency, subsequent irradiation and adjuvant chemotherapy are added to improve survival rates. Emanating from treatment strategies in metastatic disease, various new agents with more specific targets have supplemented the treatment armamentarium in recent years. Among these are small molecules inhibiting single or multiple pathways important in tumor cell proliferation, agents with antiangiogenic properties and monoclonal antibodies binding to tumor-associated targets on the cell surface and inducing immunologically mediated tumor cell lysis.

Among the different approaches to eliminate tumor cells by immunological means is active specific immunization. Provided that the target is truly cancer-specific, treatment should be virtually devoid of adverse effects, and the induction of long-term immunity could potentially eradicate minimal residual disease, averting relapse. Despite the identification of numerous more or less specific tumor-associated antigens (TAAs) (1), tumor vaccination has so far not yielded the impressive effects that have been seen for the vaccination of various infectious diseases for many decades. For example, a meta-analysis of various active specific vaccinations against metastatic colorectal cancer revealed an average objective clinical response rate of only 0.9% (2).

There are several ways of delivering antigen-derived epitopes to the immune system of cancer patients, including: 1) a sole peptide vaccine; 2) a peptide vaccine with auxiliary proteins (*e.g.*, granulocyte-macrophage colony-stimulating factor [GM-CSF]) and co-stimulatory factors (*e.g.*, keyhole limpet hemocyanin [KLH]); 3) dendritic cells (DCs) pulsed with peptides; 4) using vectors such as viruses or plasmids encoding for epitopes or whole antigens; 5) DCs transfected with epitope-encoding viruses or plasmids; and 6) whole tumor cell vaccines.

Many novel cancer vaccines have progressed to various stages of clinical testing in recent years, including whole-cell vaccines (*e.g.*, sipuleucel-T, GVAX and Onyvax-P for prostate cancer), DC-based vaccines, vector-based vaccines (*e.g.*, TG4010 encoding MUC1 and IL-2 or PSA-TRICOM encoding prostate-specific antigen [PSA] and three co-stimulatory factors for prostate cancer, CEA-TRICOM for gastrointestinal cancers and canarypox-based vectors [ALVAC] encoding melanoma targets or carcinoembryonic antigen [CEA]), various protein and peptide vaccines (either autologous, allogeneic or recombinant), as well as anti-idiotype monoclonal antibodies. The aim of these approaches is the induction of a sufficient tumor-directed immune response, defined by induction of a TAA-directed T-cell response or the detection of tumor-specific antibodies. It is widely believed that a clinically effective tumor-directed T-cell response will be the key for successful immunization. However, although there are reports of a correlation between the induction of a TAA-directed T-cell response by vaccination and clinical response (3-6), most studies report strong T-cell

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response induction without clinical response. In fact, again in metastatic colorectal cancer, with a clinical response rate of < 1%, an immune response was seen in approximately 50% of patients (2). Moreover, TAA-directed T-cell responses can already be detected in several patients naïve to immunotherapy (7). The actual functional role of these naturally arising T-cell responses has not yet been defined. It may well be that they exhibit different immune functions than vaccine-induced T-cells.

The human oncofetal antigen 5T4 is a 72-kDa membrane glycoprotein that is expressed at high levels on the placenta, as well as on a wide range of human carcinomas, including colorectal, gastric, renal and ovarian carcinomas (8-10), whereas it shows only restricted expression on normal tissues. Overexpression on tumor cells has been associated with metastatic spread and poor prognosis (11-15), although the exact role of 5T4 in oncogenesis is unclear. Due to its specificity for tumor tissue and its cell-surface expression, 5T4 was selected as a candidate antigen for the development of active immunotherapy.

Subsequently, a modified vaccinia Ankara (MVA) virus vector was engineered to encode 5T4 (TroVax®), which was further improved in 5T4 production by promoter modification and subsequently investigated both in animals and early-phase clinical trials (16-19).

Preclinical Pharmacology

Mulryan *et al*. (16) constructed a recombinant vaccinia virus based on the highly attenuated and modified vaccinia Ankara (MVA) strain expressing human 5T4 (MVA-h5T4). Their data showed that immunization of mice with these constructs induced antibody responses to 5T4 without signs of autoimmune toxicity. Furthermore, mice treated with MVA-h5T4 showed significant tumor growth delay. These preclinical data were supported by further studies. The prototype MVA-h5T4 was further improved by placing h5T4 under the control of a different promoter (mH5) and by nucleotide removal from the 5'-untranslated region, resulting in enhanced *in vitro* h5T4 expression. In another murine model, vaccination with the novel recombinant virus produced protection against a challenge with CT26-h5T4, a 5T4-expressing syngeneic colon carcinoma cell line, for up to 6 months after the final vaccination (17). In a therapeutic setting, injection of TroVax® was able to reduce tumor burden by > 90% in the mice. Whereas depletion of $CD8^+$ T-cells prior to vaccination had no effect on activity in the active treatment model, depletion of $CD4^+$ T-cells completely abrogated the immune response. Moreover, in the prophylactic setting, depletion of $CD8^+$ and $CD4^+$ T-cells did not interfere with protection from tumor challenge. These findings suggested that the effector arm was $CD4^+$ T-cell-dependent and antibody-mediated in this model. This concept was supported by an experiment in which the application of polyclonal anti-5T4 serum from vaccinated animals was able to effectively decrease tumor burden in tumor-challenged animals without vaccination.

Clinical Studies

TroVax® was evaluated in several common malignant diseases. It was first clinically investigated in humans in a phase I/II trial in 22 patients with stage IV colorectal carcinoma. The vaccine was found to be well tolerated and induced a specific immune response in the majority of patients –a 5T4-specific cellular response in 16 of 17 evaluable patients and detectable antibody levels in 14 of 17 patients. Disease stabilization for up to 18 months was achieved in 5 patients, with a positive association between the development of a 5T4 antibody response and time to disease progression (18).

The next step was to study the effects of the vaccine in combination with chemotherapy. Given the importance of chemotherapy in the adjuvant as well as palliative treatment of colorectal carcinoma, a potential attenuation of immunotherapeutic efficacy had to be ruled out. In a phase II study in 17 patients with advanced colorectal carcinoma, TroVax® was administered 4 weeks before chemotherapy with 5-fluorouracil (5-FU), leucovorin and oxaliplatin, repeatedly during the course of treatment (weeks 11 and 17) and twice after completion of chemotherapy (weeks +2 and +6). Again, the vaccination proved to be safe and well tolerated without serious adverse events attributable to TroVax®. Antibody responses, measured by ELISA, and 5T4-specific interferon gamma (IFN-γ) enzyme-linked immunospot (ELISPOT) responses could be detected in 10 of 11 evaluable patients (19). The immune responses were of greater magnitude and longer lasting than those detected in previous monotherapy trials with TroVax®. In a similar phase II trial, 19 patients received the vaccine in conjunction with 5-FU, leucovorin and irinotecan. While publication of this study is pending, a preliminary report at the ASCO 2006 meeting (20) suggested equally good tolerability and the induction of a specific cellular and/or humoral immune response.

In order to identify a potential natural 5T4-directed $CD8^+$ T-cell repertoire in healthy individuals, $CD4^+$ T-cell-depleted peripheral blood mononuclear cells (PBMCs) from 30 blood donors were studied using an ELISPOT assay with a panel of overlapping peptides spanning the full length of the 5T4 protein. Within this population, an HLA-Cw7-restricted minimal CD8 epitope of 5T4 (p8.7) could be identified in 1 healthy donor. In subsequent experiments, HLA-Cw7-positive patients from the above-mentioned interventional studies were able to mount a strong IFN-γ ELISPOT response to this novel cytotoxic T-lymphocyte epitope. These findings may have an impact on further subunit vaccine designs and monitoring of specific immune responses to TroVax® immunization (21).

Several clinical trials are ongoing or have just completed recruitment. Most clinical data are preliminary but the vaccine was generally well tolerated. Four early-phase clinical trials have been registered for the treatment of advanced or metastatic renal cell carcinoma (RCC) together with interleukin-2 (IL-2) or interferon alfa (IFN-α) therapy.

Preliminary data from two trials have been presented as abstracts. Cao *et al*. (22) reported on 41 RCC patients receiving MVA-5T4, 8 as monotherapy and 33 in combination with low-dose IL-2 or IFN. Reportedly, 5 of 36 evaluable patients had clinical responses (14%: 2 CR and 3 PR) and 8 patients (22%) had stable disease for more than 3 months. Additionally, data suggested a relationship between the anti-5T4 immune response and tumor response. Kaufman *et al*. reported preliminary data at the 2006 ASCO meeting (23) on 25 patients with RCC receiving TroVax® plus high-dose IL-2. All of these patients had developed an increase in 5T4-specific antibody titers after vaccination plus high-dose bolus IL-2. Data on cellular response and clinical benefit are pending.

A phase II trial in stage IV colorectal cancer with resectable liver metastases using TroVax® as an adjuvant to surgery has completed recruitment. Dangoor *et al*. reported at the 2006 ASCO meeting preliminary data on 20 patients, 16 of whom were eligible for clinical and immunological assessment. According to T-cell proliferation assays, 12 of 16 patients showed a cellular immune response and 14 of 16 patients developed 5T4-specific antibody responses. At a median follow-up of 8.4 months, 7 of 16 patients had recurrent disease (24).

Hormone-refractory prostate cancer is the third malignancy treated with TroVax® in a phase II trial, either as a single agent or with auxiliary GM-CSF administration. Again, the vaccination was well tolerated and able to induce immune responses (25).

Several phase II trials are ongoing evaluating TroVax® in combination with IFN-α or IL-2 in patients with advanced or metastatic RCC (26-28), and in combination with docetaxel in patients with progressive hormone-refractory prostate cancer (29).

The only ongoing phase III trial, the TroVax Renal Immunotherapy Survival Trial (TRIST) (30), is a placebo-controlled study evaluating TroVax® combined with standard treatment (IL-2, IFN-α or sunitinib) in advanced or metastatic RCC. Seven hundred patients are planned to be evaluated, primarily for overall survival. The study successfully passed the first interim data safety monitoring board analysis in July 2007 (31). Other trials in metastatic breast cancer and particularly as adjuvant treatment for stage II/III colorectal carcinoma are planned (source: www.oxfordbiomedica.co.uk).

Conclusions

Compared with other novel therapeutic approaches, TroVax® is advanced in clinical testing. So far, administration of more than 700 doses of TroVax® has been reported in more than 200 patients with various malignancies. The administration of TroVax® appears to be safe and well tolerated in various combinations, including IL-2, IFN-α, GM-CSF, FOLFOX (fluorouracil, leucovorin, oxaliplatin) and IFL (irinotecan, fluorouracil, leucovorin). 5T4-specific antibodies and robust 5T4-directed T-cell responses can be induced with all reported therapeutic approaches and the frequencies of humoral and T-cell responses exceed those observed in other cancer vaccine trials (7). Thus, TroVax® appears to have a strong immunological potential. Encouraging clinical results have been reported, particularly in advanced RCC. Specific immune responses were recorded in most patients, but a correlation with clinical benefit cannot be made due to the small patient numbers. In accordance with the opinion expressed in a recent review (32), we believe that the trend observed for a correlation between an immune response against 5T4 and clinical response might be influenced by a number of unmeasured factors, such as clinical performance status. Nonetheless, quantification of the resulting immune response, *e.g.*, 5T4 antibody levels, might be of importance for the prediction of clinical outcome. Clinical response is difficult to assess in RCC due to a variable biological disease course. The ongoing phase III trial will therefore need to demonstrate equality or even superiority in terms of progression-free and overall survival compared with standard therapy. Even then, the role of TroVax® among the broad spectrum of drugs licensed for the treatment of RCC needs to be determined. Based on the data published so far, it is likely that combination treatment with established agents will be the key for enhanced survival.

TroVax® vaccination is a promising immunotherapeutic approach worthy of further investigation. However, several other tumor vaccine approaches have shown promising immunological results which did not translate into clinical responses. Therefore, we will have to await results of larger ongoing trials to draw firm conclusions on the role of TroVax® in future cancer therapy.

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