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Vaccination of Metastatic Renal Cancer Patients with MVA-5T4: A Randomized, Double-Blind, Placebo-Controlled Phase III Study

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Abstract

Purpose: The TroVax Renal Immunotherapy Survival Trial was a randomized, placebo-controlled phase III study that investigated whether modified vaccinia Ankara encoding the tumor antigen 5T4 (MVA-5T4) prolonged survival of patients receiving first-line standard-of-care (SOC) treatment for metastatic renal cell cancer.

Experimental Design: Patients with metastatic clear cell renal cancer, prior nephrectomy, and good or intermediate prognosis were randomized 1:1 to receive up to 13 immunizations of MVA-5T4/placebo in combination with either sunitinib, interleukin-2 or interferon-α. The primary end point was overall survival. Secondary end points included progression-free survival, overall response rate, and safety.

Results: Seven hundred thirty-three patients were recruited (365 MVA-5T4 and 368 placebo). Treatment arms were well balanced for SOC and prognosis. No significant difference in the incidence of adverse events or serious adverse events was observed. No significant difference in overall survival was evident in the two treatment arms (median 20.1 months MVA-5T4 versus 19.2 months placebo; $P = 0.55$). The magnitude of the 5T4-specific antibody response induced by vaccination with MVA-5T4 was associated with enhanced patient survival. Furthermore, exploratory analyses suggested a number of pretreatment hematologic factors that could identify patients who derive significant benefit from this vaccine.

Conclusion: MVA-5T4 in combination with SOC was well tolerated, but no difference in survival was observed in the overall study population. Exploratory analyses indicate that there may be subsets of patients who could gain significant benefit from MVA-5T4, but such results would need to be confirmed in future randomized clinical studies. *Clin Cancer Res; 16(22); 5539–47. ©2010 AACR.*

Renal cell carcinoma (RCC) represents 5% of epithelial cancers diagnosed annually in the United States, the majority being of clear cell histology (1, 2). Approximately 20% to 30% of RCC patients present with metastatic disease, which has a poor prognosis. Conventional cytotoxic chemotherapeutic agents and hormonal therapies have little impact on survival, and response rates are usually <10%. Until recently, cytokine therapy using interleukin-2 (IL-2) or interferon-α (IFN-α) was the mainstay of treatment; however, it yielded low response rates of <30% and median survival times of ~1 year (3).

However, a better understanding of the mechanisms underlying RCC tumorigenesis has led to the development of new targeted agents. Indeed, the Food and Drug Administration has recently approved sunitinib, sorafenib, temsirolimus, everolimus, pazopanib, and bevacizumab for use against advanced RCC. Despite these advances, the management of metastatic RCC remains a challenge. The ability to add a new therapeutic moiety to an existing therapy without increasing toxicity or reducing efficacy would be valuable. Cancer vaccines offer one possible approach to achieve this goal. Although there have been several failed phase III trials, a number of cancer vaccine/immunotherapy products have shown encouraging data in late-stage clinical studies and the Food and Drug Administration has recently approved the first therapeutic vaccine (Provenge) for the treatment of prostate cancer. Even with this encouraging breakthrough, it is critical that we continue to gain a better understanding of the nature of the efficacious

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Translational Relevance

The TroVax Renal Immunotherapy Survival Trial is the first phase III study that aimed to establish the efficacy of TroVax (MVA-5T4) when administered alongside interleukin-2, interferon-α, or sunitinib in patients with renal cell carcinoma. The vaccine was well tolerated, but did not result in enhanced survival relative to placebo controls. Despite this equivocal result, it is important for the cancer vaccine community to learn as much as possible from the data available to aid in the design of better studies and to help identify patients who are more likely to benefit from this class of therapy. Exploratory analyses have identified subgroups of patients for whom MVA-5T4 may be of significant benefit and showed that 5T4 antibody response is associated with enhanced survival. These observations need to be tested prospectively in future studies.

immune response induced by vaccination, determine how best to monitor the response, and, ultimately, learn how to improve upon it.

The attenuated vaccinia virus modified vaccinia Ankara (MVA) has been engineered to deliver the tumor antigen 5T4 (MVA-5T4; TroVax). The 5T4 oncofetal antigen is rarely detected in normal adult tissues but is expressed at high levels in the placenta (4, 5) and in most common tumors, typically >80% of carcinomas of the kidney, breast, colon/rectum, prostate, and ovary (5, 6). Recently, a study in renal cancer showed that 5T4 was expressed at high levels in practically all tumors analyzed and expression was retained in metastatic tissues (7). Importantly, 5T4 is expressed on the cell surface, which makes it a potential target for both T-cell and antibody-mediated effector responses.

MVA-5T4 has been tested in nine phase I/II clinical trials in colorectal, renal, and prostate cancer patients. These studies showed MVA-5T4 to be well tolerated and able to induce 5T4-specific immune responses in most patients. Furthermore, associations between 5T4-specific cellular or humoral responses and clinical benefit were reported in seven of the nine studies (8–16).

Here, we report the results of a phase III randomized, double-blind, placebo-controlled study, which investigated whether MVA-5T4 could prolong survival of patients with metastatic RCC.

Patients and Methods

Eligibility criteria

The study population consisted of patients with histologically proven clear cell renal cancer who had undergone

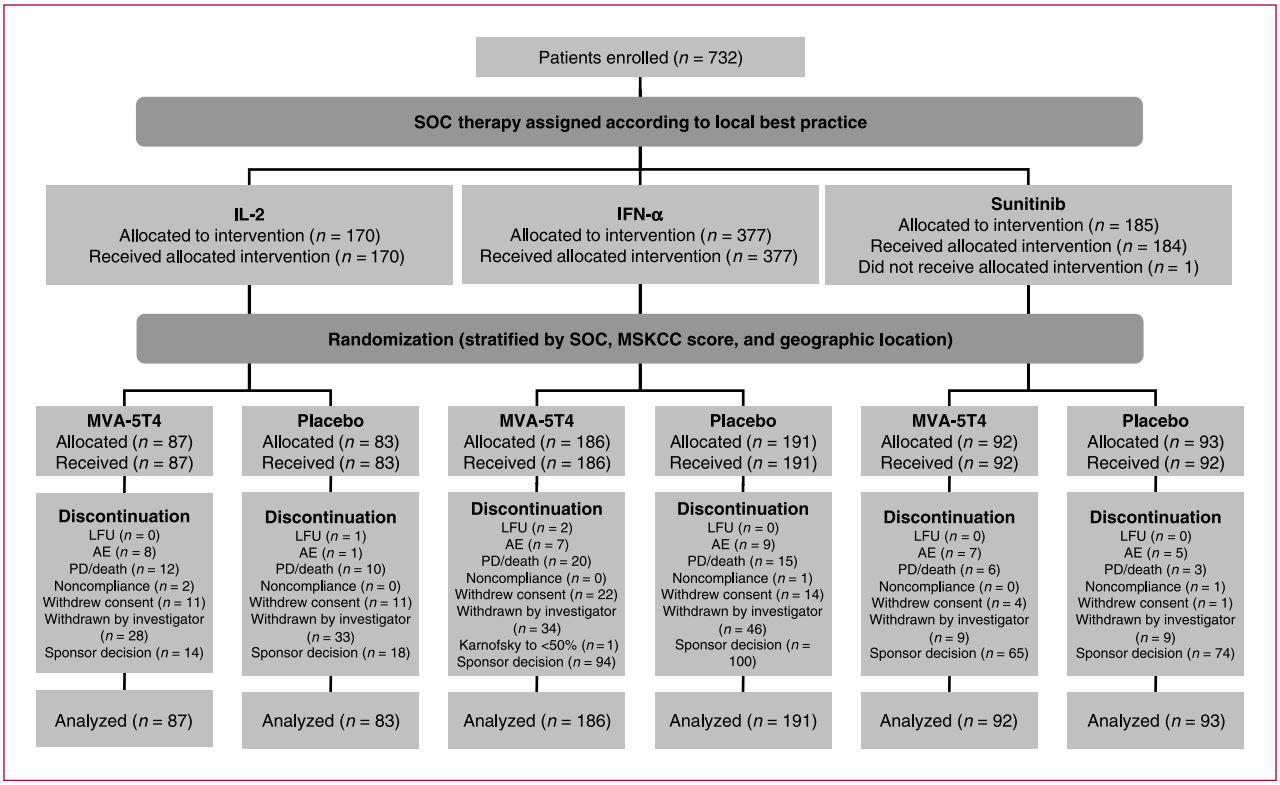

Fig. 1. CONSORT diagram. LFU, lost to follow-up; AE, adverse event; PD, progressive disease.

Table 1. Patient demographics and baseline characteristics

Characteristic	MVA-5T4 (n = 365) No. (%)	Placebo (n = 367) No. (%)	Total (%)
Age (y)			
Median	58	58	58
Range	18-86	24-85	18-86
>65	96 (26.4)	100 (27.2)	196 (26.8)
Sex			
Female	111 (30.4)	128 (34.9)	239 (32.7)
Male	254 (69.6)	239 (65.1)	493 (67.3)
Location			
United States	19 (5.2)	18 (4.9)	37 (5.0)
European Union	44 (12.1)	48 (13)	92 (12.6)
Eastern Europe	302 (82.7)	302 (82.1)	604 (82.4)
BMI			
Median	26.4	26	
>30	76 (21.2)	90 (25.1)	166 (23.1)
Karnofsky performance status			
Missing	0 (0)	1 (0.3)	1 (0.14)
80	120 (32.9)	101 (27.5)	221 (30.2)
90	154 (42.2)	165 (45)	319 (43.6)
100	91 (24.9)	100 (27.2)	191 (26.1)
Motzer (MSKCC) grade			
Favorable	211 (57.8)	214 (58.3)	425 (58.1)
Intermediate	154 (42.2)	152 (41.4)	306 (41.8)
Poor	0 (0)	1 (0.3)	1 (0.13)
No. of coded organs involved			
0	15 (4.1)	12 (3.3)	27 (3.7)
1	113 (31)	121 (33.2)	234 (32.1)
2	147 (40.3)	145 (39.7)	292 (40.0)
≥3	90 (24.7)	87 (23.8)	177 (24.2)
Prior therapy			
Surgery	365 (100)	366 (99.7)	731 (99.9)
Radiotherapy	43 (11.8)	44 (12)	87 (11.9)

Abbreviation: BMI, body mass index.

prior nephrectomy and required first-line treatment for locally advanced or metastatic disease. Patients had to be ≥18 years and have measurable disease, a Karnofsky performance status of ≥80%, a Memorial Sloan-Kettering Cancer Center (MSKCC) performance status of 0 to 2 (17), and life expectancy of >12 weeks. Patients were ineligible if they had cerebral metastases, prior exposure to MVA-5T4, had a known allergy to vaccinia vaccinations or egg proteins, or were pregnant.

Study design

The TroVax Renal Immunotherapy Survival Trial (ClinicalTrials.gov Identifier: NCT00397345) was a randomized, double-blind, placebo-controlled study that recruited patients at 111 centers in France, Germany, Israel, Poland, Romania, Russia, Spain, United Kingdom, Ukraine, and the United States. The study was approved by the relevant ethics committee or institutional review board and complied with the provisions of the Declaration of Helsinki, Good Clinical Practice guidelines, and the laws and regulations of the country in which the research was conducted. All patients gave full written informed consent before study entry. Subsequently, patients were assigned by their physician to one of the following standard-of-care (SOC) regimens consistent with local practice: (*a*) s.c. low-dose IL-2 [an initial dose of 250,000 U/kg/dose (with an upper limit of 22 million units/dose) for 5 days out of 7 in week 1 of each cycle followed by 125,000 U/kg/dose (with an upper limit of 11 million units/dose) for 5 days in each of weeks 2 to 6 of each cycle], (*b*) IFN-α (s.c. injection three times per week on days 1, 3, and 5 of each week and at a dose level that reflected local practice but was targeted between 9 and 18 million IU), or (*c*) sunitinib (50 mg oral dose taken once daily on a schedule of 4 weeks on treatment and 2 weeks off).

Treatment (MVA-5T4/placebo in a 1:1 ratio) was allocated based on a randomization that was stratified to ensure that the two arms were balanced for SOC, severity of disease (defined by MSKCC score), and geographic location. MVA-5T4 (1×10^9 $TCID_{50}$/mL) or placebo was scheduled to be administered by i.m. injection into the deltoid muscle at weeks 1, 3, 6, 9, 13, 17, 21, 25, 33, 41, 49, 57, and 65 (Supplementary Fig. S1). Placebo was produced by undertaking a mock preparation of MVA-5T4 but without the addition of virus. An identical biomanufacturing process was used (minus the virus infection steps), and the final product was formulated in the same mannitol-based formulation buffer. During the course of the study, plasma samples were obtained from patients before treatment and following the third and fourth MVA-5T4/placebo vaccinations (baseline, weeks 7 and 10, respectively) for assessment of MVA and 5T4-specific antibody responses as described previously (12).

Statistical analysis

The primary efficacy end point was overall survival (OS) in the intent-to-treat (ITT) population. Secondary end points included progression-free survival at week 26 and response rate. The primary safety end point was the incidence of adverse events in the ITT population.

The study had 80% power to detect a hazard ratio in favor of MVA-5T4 of 0.725 using two-sided tests at the 5% level of significance. Time to event analyses were done using the Kaplan-Meier method and Cox proportional hazards (PH) model with two-sided 95% confidence intervals (95% CI) for the medians and hazard ratios (HR) for each end point.

Exploratory analyses

To investigate potential imbalances between treatment arms (MVA-5T4 or placebo), a Cox PH model was fitted adjusting for known prognostic factors. The model was fitted initially by including all known prognostic variables,

and a stepwise selection procedure was then used to select the most significant variables for inclusion ($P \leq 0.1$ selection criterion used).

Results

Patient characteristics and disposition

Between October 2006 and March 2008, 733 patients were recruited, of which 732 were included in the ITT population (Fig. 1). Demographic and other baseline characteristics are summarized by treatment group in Table 1. Patient characteristics were generally well balanced between MVA-5T4 and placebo arms for SOC (IL-2: 23.8%/22.6%; IFN-α: 51.2%/51.9%; sunitinib: 24.9%/25.5%) and good prognosis (MSKCC score 0; 57.8%/58.3%).

Safety

Treatment-emergent adverse events (TEAE) were reported for 338 (92.6%) MVA-5T4 patients and 347 (94.8%) placebo patients, whereas the total number of TEAEs was 2,390 and 2,403, respectively. For both treatment groups, most of the TEAEs were of mild/moderate intensity, with only 11.4% of TEAEs in National Cancer Institute Common Toxicity Criteria grades 3 to 5. TEAEs that were life threatening or caused death were 1.7% and 2.0% of the total number reported for MVA-5T4 and placebo patients, respectively.

Serious adverse events were reported for 72 (19.7%) MVA-5T4 patients and 76 (20.8%) placebo patients (Table 2). Among the SOC subgroups, the proportion of patients with events was higher in the sunitinib subgroup than in the IL-2 or IFN-α subgroups for all categories except deaths and withdrawals due to TEAEs.

Efficacy results

At the recommendation of the data safety monitoring board, the sponsor terminated the administration of MVA-5T4/placebo to patients in July 2008 because there was little or no prospect of demonstrating a significant survival benefit. However, patient follow-up was continued. At the time of stopping the study drug, median time on study was 6 months and only 5% of patients had received the complete course of injections ($n = 13$). The median number of MVA-5T4/placebo vaccinations received was 8 for patients treated with IFN-α and IL-2 and 7 for patients treated with sunitinib.

The survival data reported here were collected by active follow-up and censored to March 2009. Follow-on therapies were well balanced by treatment arm [32% placebo

Table 2. Treatment-emergent serious adverse events

Adverse event	MVA-5T4				Placebo			
	Grade				Grade			
	All No. (%)	3 No. (%)	4 No. (%)	5 No. (%)	All No. (%)	3 No. (%)	4 No. (%)	5 No. (%)
All	72 (19.7)	34 (9.3)	17 (4.7)	8 (2.2)	76 (20.8)	30 (8.2)	19 (5.2)	9 (2.5)
Blood and lymphatic	17 (4.7)	8 (2.2)	6 (1.6)	0 (0)	14 (3.8)	4 (1.1)	9 (2.5)	0 (0)
Cardiac disorders	3 (0.8)	1 (0.3)	2 (0.5)	0 (0)	2 (0.5)	1 (0.3)	0 (0)	1 (0.3)
Congenital, familial, and genetic	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.3)	1 (0.3)	0 (0)	0 (0)
Ear and labyrinth	1 (0.3)	1 (0.3)	0 (0)	0 (0)	1 (0.3)	0 (0)	0 (0)	0 (0)
Endocrine	1 (0.3)	0 (0)	0 (0)	0 (0)	1 (0.3)	0 (0)	0 (0)	0 (0)
Gastrointestinal	17 (4.7)	9 (2.5)	1 (0.3)	0 (0)	14 (3.8)	8 (2.2)	1 (0.3)	0 (0)
General disorders	14 (3.8)	7 (1.9)	0 (0)	2 (0.5)	11 (3)	3 (0.8)	1 (0.3)	2 (0.5)
Hepatobiliary	1 (0.3)	0 (0)	0 (0)	1 (0.3)	1 (0.3)	1 (0.3)	0 (0)	0 (0)
Immune system	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.3)	1 (0.3)	0 (0)	0 (0)
Infections and infestations	7 (1.9)	4 (1.1)	1 (0.3)	2 (0.5)	7 (1.9)	2 (0.5)	1 (0.3)	2 (0.5)
Injury poisoning and procedural	2 (0.5)	2 (0.5)	0 (0)	0 (0)	3 (0.8)	2 (0.5)	0 (0)	0 (0)
Investigations	2 (0.5)	1 (0.3)	1 (0.3)	0 (0)	4 (1.1)	3 (0.8)	0 (0)	0 (0)
Metabolism and nutrition	4 (1.1)	1 (0.3)	1 (0.3)	0 (0)	5 (1.4)	3 (0.8)	1 (0.3)	0 (0)
Musculoskeletal and connective tissue	5 (1.4)	5 (1.4)	0 (0)	0 (0)	8 (2.2)	6 (1.6)	0 (0)	0 (0)
Neoplasms benign, malignant, and unspecified	5 (1.4)	1 (0.3)	1 (0.3)	0 (0)	12 (3.3)	2 (0.5)	5 (1.4)	0 (0)
Nervous system	13 (3.6)	5 (1.4)	4 (1.1)	1 (0.3)	10 (2.7)	3 (0.8)	3 (0.8)	1 (0.3)
Psychiatric	3 (0.8)	1 (0.3)	0 (0)	0 (0)	1 (0.3)	1 (0.3)	0 (0)	0 (0)
Renal and urinary	3 (0.8)	2 (0.5)	0 (0)	0 (0)	4 (1.1)	2 (0.5)	1 (0.3)	0 (0)
Respiratory, thoracic, and mediastinal	7 (1.9)	3 (0.8)	1 (0.3)	1 (0.3)	13 (3.6)	4 (1.1)	1 (0.3)	2 (0.5)
Vascular	1 (0.3)	1 (0.3)	0 (0)	0 (0)	2 (0.5)	1 (0.3)	1 (0.3)	0 (0)

NOTE: The total number (and percentage) of adverse events are listed by system organ class and grade.

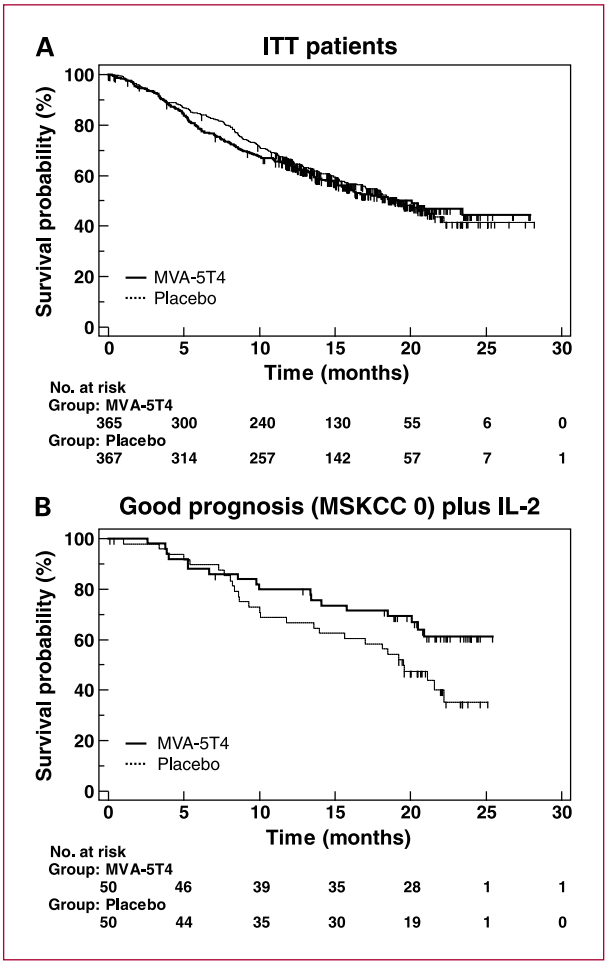

Fig. 2. Kaplan-Meier survival plots of the ITT patient population (A) and patients with a good prognosis (MSKCC 0) receiving IL-2 (B) stratified by treatment arm (solid line, MVA-5T4; dotted line, placebo).

patients (n = 119) and 29% MVA-5T4 patients (n = 106)], the most common being sorafenib or radiotherapy. However, the incidence of treatment with second-line therapies was higher in patients who received sunitinib (42%) compared with those who received IFN-α (28%) or IL-2 (24%), and similarly in the United States (59%) compared with Eastern (29%) or Western (47%) Europe. At March 2009, median follow-up was 12.9 months and a total of 331 events had occurred, which represented 45.2% (n = 165) of MVA-5T4–treated and 45.2% (n = 166) of placebo-treated patients. Median OS was 20.1 and 19.2 months for MVA-5T4 and placebo-treated patients, respectively (Fig. 2A; HR, 1.07; 95% CI, 0.86-1.32; P = 0.55). Treatment and geographic region did not have a statistically significant effect on the risk of death in the ITT population. Analysis of patient subsets classified by prognostic index (MSKCC grade) or SOC showed no significant differences in survival outcome (data not shown). However, patients with a good prognosis (MSKCC 0) and treated with MVA-5T4 plus IL-2 showed a significant survival advantage compared with good-prognosis patients receiving placebo plus IL-2 (Fig. 2B; HR, 0.54; 95% CI, 0.30-0.98; P = 0.046). No other prospectively defined strata showed a significant difference in OS.

Computed tomography scans were done at week 26, and complete responses (MVA-5T4, n = 2; placebo, n = 5), partial responses (MVA-5T4, n = 47; placebo, n = 46), or stable disease were observed in 164 (44.9%) MVA-5T4–treated patients and 173 (47.1%) placebo-treated patients.

Although the study was not designed or powered to detect differences between SOC treatment groups, it was of interest to compare the survival of patients treated with IFN-α, IL-2, or sunitinib (Table 3). Placebo patients with a good prognosis (Fig. 3A) and treated with either sunitinib or IFN-α showed comparable survival, both of which were superior to IL-2–treated patients. In contrast, there was no significant difference in the survival of patients who had a good prognosis (Fig. 3B) and were treated with MVA-5T4 plus sunitinib compared with those receiving IFN-α or IL-2; the median survival has not been reached for any of these three SOC groups.

Analysis of OS in intermediate-prognosis, placebo patients (Fig. 3C) suggested that those receiving sunitinib had a favorable median survival of 18 months compared with 9.2 months for patients treated with IL-2 or 10.6 months for IFN-α. Intermediate-prognosis patients treated with MVA-5T4 plus sunitinib also showed a favorable median survival (not reached; Fig. 3D) compared with those treated with IL-2 or IFN-α.

Exploratory multivariate analyses

The influence of baseline clinical features and known prognostic risk factors (some of which were not prespecified in the study protocol) on OS was analyzed using a Cox PH model. In developing the Cox model, 10 prognostic variables were selected, in addition to treatment, using a $P \leq 0.1$ significance inclusion criterion (SOC, age, body mass index, gender, number of metastatic sites, corrected calcium, hemoglobin, lactate dehydrogenase, alkaline phosphatase, and neutrophils). In total, 702 patients and 313 events were included in the final model (31 patients and 18 events were excluded from the model due to missing hematology data). After adjusting for prognostic factors, the HR for treatment decreased from 1.07 before adjustment to 0.97 (Fig. 4A; 95% CI, 0.78-1.22; P = 0.82). The linearity assumption for continuous variables, the PH assumption, and the overall model fit were checked and found to be satisfactory.

Additional exploratory analyses investigated the role of pretreatment hematologic factors on the relative efficacy of MVA-5T4. It was noted that baseline levels of platelets, monocytes, and hemoglobin seemed to affect the relative efficacy of MVA-5T4. Indeed, patients who presented with normal levels of platelets ($\leq 400 \times 10^9$/L), monocytes ($\leq 0.80 \times 10^9$/L), and hemoglobin (age/gender-specific ranges) had an adjusted HR of 0.72 favoring the MVA-5T4 arm (Fig. 4B; 95% CI, 0.48-1.08; P = 0.109).

Analysis of antibody responses

Antibody responses against 5T4 and MVA were quantified at two posttreatment time points (post third and fourth vaccination). At these snapshots in time, positive MVA and 5T4-specific antibody responses were detected in 96% and 56% of MVA-5T4–treated patients and 5% and 6% of placebo-treated patients, respectively. 5T4 seroconversion rates were higher in MVA-5T4 patients treated with IFN-α than in those treated with IL-2 and sunitinib. There was no difference in seroconversion rate in patients classified as having intermediate or good prognosis (data not shown).

Exploratory analyses were undertaken to determine whether 5T4-specific antibody responses were associated with enhanced patient survival. Two exemplary subgroups of 50 MVA-5T4–treated individuals were constructed using patients showing the greatest increase (>4-fold relative to pretreatment levels) in 5T4 antibody response and patients showing the greatest increase (≥15-fold) in MVA antibody response, respectively, both post fourth vaccination (week 10). Survival estimates for the high 5T4 antibody responding subset compared with placebo patients (who survived until at least week 10; $n = 303$) are illustrated in Fig. 5A, where it can be seen that the high 5T4 antibody responders showed a favorable survival compared with placebo-treated patients (HR, 0.55; 95% CI, 0.39-0.97). In comparison, the high MVA antibody responding subset did not show a favorable survival compared with placebo-treated patients (Fig. 5B; HR, 1.30; 95% CI, 0.82-2.12). These figures illustrate the association of high 5T4 antibody response with survival; however, direct comparisons with placebo should be made with caution because the plotted subgroups are not balanced with respect to confounding factors such as SOC.

Discussion

The 5T4 oncofetal antigen is a promising target for a cancer vaccine due to its surface expression, its presence on most solid tumors, and its apparent role in disease progression. Results from phase I and II clinical studies of MVA-5T4 in renal, colorectal, and prostate cancer patients were encouraging and showed that immune responses were induced in almost all treated patients, and associations between 5T4-specific cellular or humoral responses and clinical benefit were reported in seven of nine studies (8–16). In particular, studies in RCC and colorectal cancer patients have detected an association between 5T4-specific (but not MVA) antibody responses and enhanced survival (9, 12, 16).

However, in this phase III study, the addition of MVA-5T4 to first-line SOC did not prolong survival of patients with metastatic RCC compared with placebo. Likewise, there was no statistically significant difference in progression-free survival at 26 weeks or objective response rates between MVA-5T4 and placebo groups. Treatment arms were shown to be well balanced for MSKCC grade and performance status. However, exploratory analyses showed small imbalances in several prognostic factors that favored the placebo arm, but these were not sufficient to have caused the failure of the study.

Unlike cytotoxic chemotherapy, the therapeutic effects of a cancer vaccine depend on multiple complex physiologic steps before the delivery of any clinical benefit. Therefore, it may take months before any changes in markers of clinical efficacy are seen. Indeed, several studies with immunotherapy agents have reported that evidence of clinical benefit was not seen until ≥9 months following treatment initiation (18, 19). In this study, treatment with

Table 3. Comparison of treatment benefit by SOC and prognostic group

Prognostic group	SOC	Patient nos.		Median OS (mo)		Comparison of SOCs	HR (0.95 CI) *P*	
		MVA-5T4	Placebo	MVA-5T4	Placebo		MVA-5T4	Placebo
Good	IL-2	50	50	NR	19.5	IL-2 vs IFN-α	1.08 (0.59-2.00) *P* = 0.80	2.05 (1.26-3.91) *P* < 0.01
	IFN-α	98	108	NR	NR	IFN-α vs sunitinib	0.82 (0.43-1.53) *P* = 0.52	1.42 (0.69-2.83) *P* = 0.35
	Sunitinib	63	56	NR	NR	IL-2 vs sunitinib	0.87 (0.42-1.75) *P* = 0.66	2.38 (1.23-5.05) *P* = 0.01
Intermediate	IL-2	37	33	5.5	9.2	IL-2 vs IFN-α	1.66 (1.10-2.85) *P* = 0.02	0.98 (0.61-1.57) *P* = 0.93
	IFN-α	88	83	11	10.6	IFN-α vs sunitinib	1.55 (0.87-2.55) *P* = 0.15	1.83 (1.07-2.82) *P* = 0.03
	Sunitinib	29	36	NR	18	IL-2 vs sunitinib	2.45 (1.35-4.46) *P* < 0.01	1.86 (1.05-3.88) *P* = 0.04

NOTE: The table details the predicted median survival, HR (0.95 CI), and *P* value of patients treated with either MVA-5T4 or placebo and subdivided by prognostic group (good or intermediate prognosis) and SOC.
Abbreviation: NR, not reached.

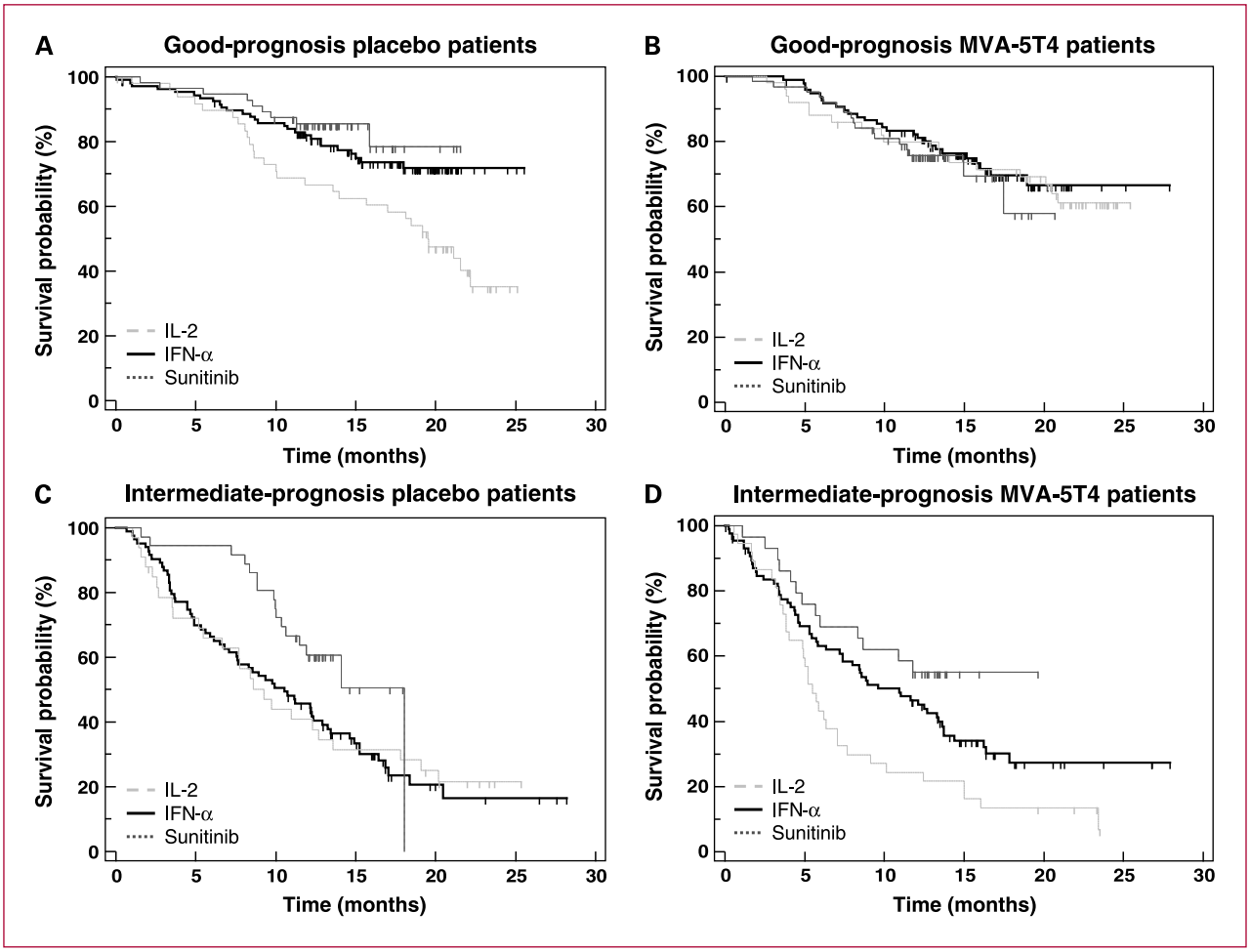

Fig. 3. Kaplan-Meier survival plots of placebo-treated (A and C) or MVA-5T4–treated (B and D) patients plotted by prognostic group [MSKCC good prognosis (A and B) or intermediate prognosis (C and D)] and stratified by SOC received (solid line, IFN-α; hashed line, IL-2; dotted line, sunitinib).

MVA-5T4 was terminated due to futility when patients had a median time on study of 6 months. It is possible that this was insufficient time to see a positive impact on patient survival, especially as so few patients received the full regimen of vaccinations.

Despite not meeting the primary end point, it is important that the phase III data are subject to extensive exploratory analyses in case, for some reason, an efficacy signal is present but not immediately apparent. The results of such analyses can be used to aid design of future clinical trials of MVA-5T4. Thus far, our exploratory analyses have focused on (*a*) the identification of patient subgroups showing treatment benefit, (*b*) the effects of baseline hematologic factors on survival, and (*c*) the effects of antibody response on survival.

(*a*) Although this study was not powered to detect survival differences in patient subgroups, it was interesting to note that patients with a good prognosis and treated with MVA-5T4 plus IL-2 showed a significant survival advantage compared with good-prognosis patients treated with placebo plus IL-2. Previous data from phase II studies in RCC patients treated with MVA-5T4 and IL-2 also showed encouraging signs of clinical benefit (13, 16). Currently, it is unclear why a survival advantage in patients with an intermediate prognosis treated with MVA-5T4 plus IL-2 was not seen. However, it was noteworthy that patients with an intermediate prognosis had a significantly elevated incidence of thrombocytosis compared with patients with a good prognosis (39% versus 8%, respectively). Furthermore, the incidence of thrombocytosis was much higher in intermediate-prognosis patients treated with IL-2 (50%) compared with those treated with IFN-α (38%) or sunitinib (29%). Patients who have thrombocytosis and are classified as having intermediate prognosis may have particularly aggressive disease and therefore insufficient time to benefit from an immunotherapy before their performance status deteriorates substantially.

(*b*) Our exploratory analyses suggested that survival of MVA-5T4–treated patients was prolonged within the subgroup of patients who had normal pretreatment levels of platelets, monocytes, and hemoglobin.

(*c*) It was encouraging to note that data from this large phase III study confirmed the association between 5T4 (but not MVA) antibody responses and enhanced patient survival already seen in phase II. This type of analysis is open to criticism because patients who mount high 5T4 antibody responses could simply be healthier patients. However, if improved survival was a function of the general health status and immune competence of a patient, it is likely that the antibody response to both 5T4 and MVA would show an association with survival; this was not the case. Furthermore, no evidence has thus far emerged from this study to suggest that patients with a better prognosis (MSKCC score of 0) mounted stronger 5T4-specific antibody responses.

Of course, it is not possible to draw definitive conclusions from unplanned exploratory analyses. At best, they can provide insights that can be used in the planning and analysis of future studies. To this end, our exploratory analyses are continuing and will be reported in due course.

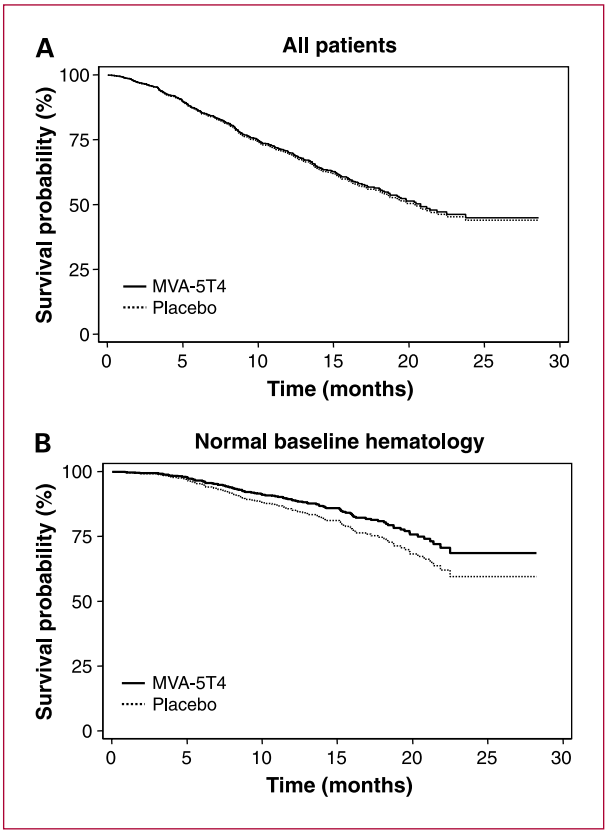

Fig. 4. Cox proportional hazard model of (A) all patients who had data available for the variables included in the model (n = 699 patients) and (B) patients with normal levels of platelets, monocytes, and hemoglobin (n = 372 patients) plotted by treatment arm (solid line, MVA-5T4; dotted line, placebo).

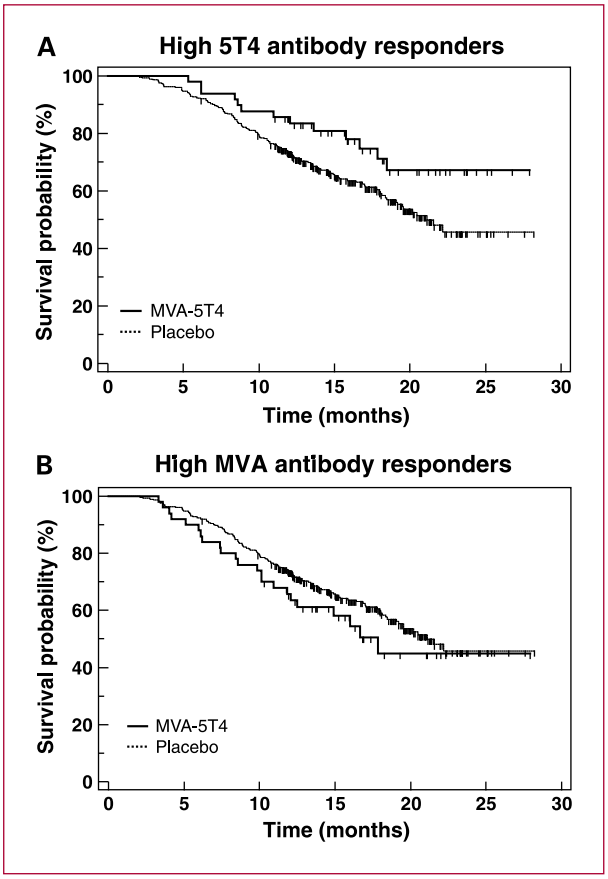

Fig. 5. Kaplan-Meier survival curves comparing MVA-5T4–treated patients (solid line) who mounted strong 5T4 (A) or MVA (B) antibody responses to placebo-treated patients (dotted line).

It is well known that cancer vaccines have an indirect mode of action, relying on activation and interaction of various elements of the immune system to achieve therapeutic efficacy. This indirect mode of action means that defining the "right patient" and identifying a predictor of treatment benefit is likely to prove challenging. It is not only expression of the target tumor antigen that determines treatment suitability but also the responsiveness of the patients' immune system as a whole and the status of the tumor microenvironment. Given these facts, it is perhaps not surprising that it will take months before treatment benefit is detectable and that hematologic factors affect the generation of robust immune responses. For these reasons, future clinical studies of MVA-5T4 will target patients with good performance status and minimize the recruitment of patients with abnormal levels of various hematology factors.

In conclusion, this study showed that MVA-5T4 was safe and well tolerated when administered in combination with IL-2, IFN-α, or sunitinib. However, the study failed to meet its primary end point of an increase in survival.

Exploratory analyses have suggested subgroups of patients for whom MVA-5T4 may be of benefit. These intimations of efficacy need to be tested prospectively in future studies.

Disclosure of Potential Conflicts of Interest

R. Harrop, W.H. Shingler, and S. Naylor are named inventors on several Oxford BioMedica patents. R.E. Hawkins has a minor consultancy role with Oxford BioMedica.

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References

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. CA Cancer J Clin 2009;59:225–49.
2. Costa LJ, Drabkin HA. Renal cell carcinoma: new developments in molecular biology and potential for targeted therapies. Oncologist 2007;12:1404–15.
3. McDermott DF. Immunotherapy of metastatic renal cell carcinoma. Cancer 2009;115:2298–305.
4. Hole N, Stern PL. A 72 kD trophoblast glycoprotein defined by a monoclonal antibody. Br J Cancer 1988;57:239–46.
5. Southall PJ, Boxer GM, Bagshawe KD, Hole N, Bromley M, Stern PL. Immunohistological distribution of 5T4 antigen in normal and malignant tissues. Br J Cancer 1990;61:89–95.
6. Starzynska T, Marsh PJ, Schofield PF, Roberts SA, Myers KA, Stern PL. Prognostic significance of 5T4 oncofetal antigen expression in colorectal carcinoma. Br J Cancer 1994;69:899–902.
7. Griffiths RW, Gilham DE, Dangoor A, et al. Expression of the 5T4 oncofoetal antigen in renal cell carcinoma: a potential target for T-cell-based immunotherapy. Br J Cancer 2005;93:670–77.
8. Elkord E, Dangoor A, Drury NL, et al. An MVA-based vaccine targeting the oncofetal antigen 5T4 in patients undergoing surgical resection of colorectal cancer liver metastases. J Immunother 2008;31:820–9.
9. Harrop R, Connolly N, Redchenko I, et al. Vaccination of colorectal cancer patients with modified vaccinia Ankara delivering the tumor antigen 5T4 (TroVax) induces immune responses which correlate with disease control: a phase I/II trial. Clin Cancer Res 2006;12:3416–24.
10. Harrop R, Drury N, Shingler W, et al. Vaccination of colorectal cancer patients with TroVax given alongside chemotherapy (5-fluorouracil, leukovorin and irinotecan) is safe and induces potent immune responses. Cancer Immunol Immunother 2008;57:977–86.
11. Amato RJ, Drury N, Naylor S, et al. Vaccination of prostate cancer patients with modified vaccinia Ankara delivering the tumor antigen 5T4 (TroVax): a phase 2 trial. J Immunother 2008;6:577–85.
12. Amato RJ, Shingler W, Goonewardena M, et al. Vaccination of renal cell cancer patients with modified vaccinia Ankara delivering the tumor antigen 5T4 (TroVax) alone or administered in combination with interferon-α (IFN-α): a phase 2 trial. J Immunother 2009;32:765–72.
13. Amato RJ, Shingler W, Naylor S, et al. Vaccination of renal cell cancer patients with modified vaccinia Ankara delivering tumor antigen 5T4 (TroVax) administered with interleukin 2: a phase II trial. Clin Cancer Res 2008;14:7504–10.
14. Harrop R, Drury N, Shingler W, et al. Vaccination of colorectal cancer patients with modified vaccinia Ankara encoding the tumor antigen 5T4 (TroVax) given alongside chemotherapy induces potent immune responses. Clin Cancer Res 2007;13:4487–94.
15. Hawkins RE, Macdermott C, Shablak A, et al. Vaccination of patients with metastatic renal cancer with modified vaccinia Ankara encoding the tumor antigen 5T4 (TroVax) given alongside interferon-α. J Immunother 2009;32:424–9.
16. Kaufman HL, Taback B, Sherman W, et al. Phase II trial of Modified Vaccinia Ankara (MVA) virus expressing 5T4 and high dose Interleukin-2 (IL-2) in patients with metastatic renal cell carcinoma. J Transl Med 2009;7:2.
17. Motzer RJ, Mazumdar M, Bacik J, Berg W, Amsterdam A, Ferrara J. Survival and prognostic stratification of 670 patients with advanced renal cell carcinoma. J Clin Oncol 1999;17:2530–40.
18. Madan RA, Arlen PM, Mohebtash M, Hodge JW, Gulley JL. Prostvac-VF: a vector-based vaccine targeting PSA in prostate cancer. Expert Opin Investig Drugs 2009;18:1001–11.
19. Hodi FS, Schmollinger JC, Soiffer RJ, et al. ATP6S1 elicits potent humoral responses associated with immune-mediated tumor destruction. Proc Natl Acad Sci U S A 2002;99:6919–24.