ILLiad TN: 427501

Lending String:

Patron: Catallo, Rosemary - 289285

Journal Title: Immunotherapy

Volume: 4 **Issue:** 1
Month/Year: 2012
Pages: 27-42

Article Author: Zhang R;Bines S;Ruby C;Kaufman H

Article Title: TroVax(®) vaccine therapy for renal cell carcinom

Imprint:

ILL Number: 32471679

ARIELBorrower: TXUTAH

Shipping Address:
University of Texas MD Anderson
Cancer Center
Research Medical Library, Unit 1499
PO Box 301402
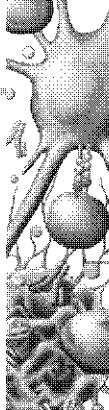Houston, TX 77230-1402
Fax: 713-563-3650
Ariel: 143.111.12.93
Odyssey IP:

MAX COST:$30.00

CHARGE: $11.00

TroVax® vaccine therapy for renal cell carcinoma

Renal cell carcinoma (RCC) is the most common primary malignancy affecting the kidney. In the past decade, several well-designed clinical trials have shifted the treatment paradigm for RCC to favor targeted therapies as first-line agents. Recognition of the immunogenic nature of RCC has also resulted in the development of immunotherapy approaches with high-dose IL-2 treatment being the best established and associated with durable disease control. The lack of defined antigens in RCC has hindered more specific vaccine development. TroVax® is a novel vaccine based on a modified vaccinia virus Ankara vector engineered to express the 5T4 tumor-associated antigen, found on over 95% of clear cell and papillary RCC tumors. The safety and efficacy of TroVax has been evaluated in several Phase I/II clinical trials and in a multicenter Phase III trial. This article will discuss the clinical background of RCC, the rationale for TroVax development, results of several TroVax clinical trials and future directions for optimizing TroVax therapy in patients with RCC and other cancers.

KEYWORDS: 5T4 ■ modified vaccinia virus Ankara ■ renal cell carcinoma ■ vaccine

Ruojiao Zhang,
Steven D Bines,
Carl Ruby &
Howard L Kaufman

Renal cell carcinoma (RCC) is a tumor of the renal epithelium and accounts for 92% of all primary tumors arising from the kidney. RCC is distinguished from less common subtypes of kidney tumors, such as transitional cell carcinoma of the renal pelvis (7%) and Wilm's tumor of childhood (1%) [1]. RCC is considered an immunogenic cancer with documented cases of spontaneous regression of metastatic lesions following nephrectomy [2]. RCC accounts for approximately 2–3% of all cancers worldwide, with the incidence rates being highest in North America, Scandinavia and western Europe [3,4]. In the USA, there are expected to be 60,920 new cases of RCC and 13,120 deaths in 2011 [1]. The median age at diagnosis is in the sixth decade and RCC is rare before the fourth decade except in patients from family clusters or with a known hereditary predisposition to the disease.

Despite increasing incidence of the disease, a trend towards improved survival has been observed in the past four decades, a phenomenon likely explained by earlier, incidental detection of asymptomatic tumors allowing for curative resection of lower-staged disease [3]. The anatomic extent of tumor growth remains the major determinant of clinical outcome. The American Joint Committee on Cancer (AJCC) assesses the extent of tumor growth and invasion via the tumor, nodes and metastasis (TNM) system. The TNM system defines stage I and II disease as tumors less than or greater than 7 cm in size, respectively, but which are confined to the kidney. These patients have a 75–90% 5-year survival rate following nephrectomy. Stage III disease is defined by extension of tumor into the inferior vena cava or renal vein, and is associated with a 5-year survival of 59–70%. Finally, stage IV disease occurs when distant lymph node or organ metastasis has occurred, at which point the 5-year survival rate drops to 20% [5]. Histopathological grade is determined by the four-tiered Fuhrman nuclear grade, and has been correlated with 5-year survival rates ranging from 86–89% (grade I) to 28–30% (grade IV) in patients with clear-cell RCC [6]. Clinical factors negatively associated with survival outcome include poor performance status and various hematologic derangements including anemia and thrombocytosis [7,8].

There are several well-defined risk factors for the development of RCC. Historically, men are at about a twofold risk of acquiring RCC compared with women [3]. Cigarette smoking is known to be a significant etiological risk factor for the development of the disease, and increases its likelihood by nearly twofold [3,9]. Other associated risk factors include obesity, hypertension [9], use of phenacetin-containing analgesics and occupational exposure to a variety of chemicals, including benzene, petroleum oil, asbestos and vinyl chloride [3,10]. Although the genetic basis of RCC is not fully understood, individuals with RCC in first-degree relatives are four-times as

Future Medicine part of

likely to develop the disease [3]. In general, the onset of RCC is in most cases sporadic with less than 3% of cases occurring through a known hereditary mechanism. An important inherited cancer syndrome to note is Von Hippel-Lindau disease (VHLD), an autosomal dominant condition predisposing to tumor development in multiple organs [11]. The primary components of VHLD include CNS and retinal hemangiomas, renal cystic disease and RCC, and pheochromocytoma. In patients with VHLD, RCC remains the leading the cause of mortality, and develops in 25–45% of cases.

Histologically, RCC is classified into clear cell, papillary, chromophobe, sarcomatoid and collecting duct variants [12]. About 75–85% of cases are clear-cell adenocarcinoma of proximal tubular origin [3]. The clear-cell variant is characterized histologically by lipid-rich epithelial cells with a clear cytoplasmic milieu nestled within a sinusoid-like, highly vascular architecture [13]. Due to the hypervascular nature of RCC, gross specimens often contain multiple areas of hemorrhage and necrosis. Up to 96% of clear-cell tumors are associated with 3p deletions in chromosome 3, which results in mutational inactivation of the Von Hippel-Lindau tumor suppressor gene. This enables the expression of various hypoxia-inducible gene products implicated in angiogenesis, including VEGF, PDGF, TGF-α and erythropoietin [3,13]. Papillary and chromophobe variants are seen in 10–15% and 4–5% of RCC patients, respectively, and are associated with a more indolent course than that of clear-cell RCC. Collecting duct RCC is an exceedingly rare and aggressive variant that is associated with transitional cell carcinoma of the renal pelvis. Sarcomatoid histologic changes may be seen in any RCC variant, and its presence is associated with a poor prognosis [14]. In general, studies have shown that nonclear cell variants of RCC respond poorly to immunotherapy [15].

Most patients with RCC present variably and, because of a lack of early warning symptoms at the onset of the disease, are frequently at an advanced stage of the disease when diagnosed. Small tumors confined to the renal parenchyma are unlikely to cause pronounced systemic effects and for this reason RCC will tend to remain undetected until invasive spread or mass effect caused by tumor growth has occurred. The classic triad of hematuria, abdominal pain and palpable flank mass occurs in less than 10% of patients and as such is not a reliable diagnostic indicator [3]. Various endocrine and hematologic symptoms suggestive of paraneoplastic disease (e.g., anemia, hypercalcemia and fever) will manifest in less than 5% of patients. Less than 3% of RCC cases present with bilateral involvement. Frequent sites of metastasis at the time of diagnosis include lungs (50–60%), bone (30–40%), liver (30–40%) and brain (<5%) [3]. The early detection of small tumors permits the greatest chance for a curative resection and, as of 2004, 57.1% of patients presented with stage I disease compared with 43% in 1993. At the same time, diagnosis of stage IV disease decreased from 27.4% of patients to 18.7% [16]. Increasingly, early stage RCC is being detected via routine imaging performed for other indications. Both CT and MRI confer excellent diagnostic accuracy in the detection of RCC [17].

The prognosis of metastatic stage IV disease has been evaluated in a retrospective study of 670 patients with RCC treated in successive clinical trials over a 21-year period [7]. The study conducted by the Memorial Sloan-Kettering Cancer Center (MSKCC) utilized a multivariate analysis of patient data to associate poor survival outcome with five pretreatment factors: low Karnofsky performance status (<80%); high lactate dehydrogenase level (>1.5-times that of the upper limit of normal); high corrected serum calcium level (>10 mg/dl); low serum hemoglobin (less than that of the lower limit of normal); and absence of prior nephrectomy. These five prognostic factors were used to stratify RCC patients into one of three risk groups based on predicted survival: favorable-risk (no risk factors); intermediate-risk (one or more risk factors); and poor-risk (three or more risk factors). 3-year survival rates among the three groups were 31, 7 and 0%, respectively. The MSKCC integrated prognostic model has since been used to reliably predict response to therapy in patients with RCC, and to determine eligibility for clinical trials.

Current therapies for RCC

Radical surgical resection of the kidney remains the mainstay treatment option for early and locally advanced disease. Spontaneous tumor regression following radical nephrectomy occurs in less than 1% of patients with distant metastasis [18]. The use of nephron-sparing surgery is controversial, but may be considered for patients with small, localized tumors with preserved renal function in both unilateral and bilateral disease, and in whom the surgery is technically feasible [19]. To date, adjuvant therapy of RCC with radiation or cytotoxic chemotherapy has not been associated with improved survival [18].

In patients with established metastatic RCC (mRCC), nephrectomy and external beam radiation therapy are both considered palliative measures. A small minority may benefit from cytoreductive surgery or metastatectomy prior to the initiation of systemic therapy. In general, mRCC is known to be highly resistant to systemic chemotherapeutic, radiotherapeutic and hormonal interventions [3,7,20]. Prior to the discovery of targeted therapies, cytokine immunotherapy with IL-2 or IFN-α was considered the standard of care for mRCC, with an overall objective response rate seen in 10–20% of patients [18,20,21]. IL-2 is a cytokine growth factor which potentiates the maturation and proliferation of T lymphocytes. High-dose recombinant IL-2 is effective in mediating the complete regression of tumors in about 5–9% of patients with mRCC [22], although therapy must often be performed at specialized centers capable of managing IL-2-related toxicities, such as vascular leak syndrome. IFN-α, another immunomodulatory cytokine, has resulted in a few cases of durable, complete response in mRCC. IFN-α in combination with IL-2 has not been shown to improve progression-free survival (PFS) or overall survival (OS) compared with IFN-α monotherapy [23].

Insights into the molecular basis of RCC and results of pivotal clinical trials have focused much interest in the development of targeted therapy for mRCC. TABLE 1 lists the US FDA-approved targeted therapies for mRCC as of June 2011. Both VEGF and PDGF are growth factors implicated in tumor angiogenesis and immune suppression for a variety of cancers, including RCC. Inactivation of the *VHL* gene has been shown to upregulate production of *HIF-1* gene expression, leading to increased VEGF and PDGF production [11]. An approach to downregulating VEGF and PDGF activity by targeting the receptor tyrosine kinases of the signaling cascade has been achieved through blocking the intracellular domain of the receptors with small molecule inhibitors or the blockade of the interaction of these molecules with their receptors through the use of monoclonal antibodies. Three orally administered small molecule tyrosine kinase inhibitors have been approved by the FDA following completed Phase III studies for use in clear-cell mRCC: sunitinib, sorafenib and more recently pazopanib. Sunitinib is a first-line tyrosine kinase inhibitor, which was found to confer improved PFS compared with IFN-α monotherapy in patients with favorable or intermediate MSKCC prognosis RCC [24]. Sorafenib is indicated as a second-line treatment following failure of first-line immunotherapy [25]. Finally, pazopanib was approved as second-line therapy after objective tumor responses and improvement in PFS were demonstrated in a Phase III study [26]. Bevacizumab is a mouse-derived, humanized monoclonal antibody that binds circulating VEGF, inhibits tumor angiogenesis and has demonstrated antitumor activity in colorectal and lung carcinomas. In 2009 bevacizumab was approved by the FDA for mRCC based on the results of a large, multicenter Phase III study demonstrating an improvement in median PFS with a combination of bevacizumab and IFN-α compared with placebo and IFN-α. In this study, adverse events associated with IFN-α therapy were reported in both groups, while an increase in grade 3 adverse events associated with bevacizumab therapy (e.g., coagulopathy, hypertension and proteinuria) contributed to a higher rate of study withdrawal in the bevacizumab arm [27].

The inhibitors of the mTOR represent a third strategy whereby production of the *HIF-1* gene product is diminished by interfering with upstream mTOR signaling. The two agents in this category, temsirolimus and everolimus, are both approved for use in mRCC. Temsirolimus is an intravenous medication that was approved as a first-line agent after efficacy was confirmed in a Phase III clinical trial which showed significant improvement in both PFS and OS when compared with IFN-α [28]. Everolimus is an orally administered mTOR inhibitor currently approved for second-line use in mRCC, and was evaluated in a Phase III crossover clinical trial in patients who had failed VEGF inhibitor therapy. Compared to the placebo arm, patients who received everolimus demonstrated an improvement in median PFS [29].

Although modest degrees of success have been seen with the use of targeted therapies in mRCC, a well-defined treatment paradigm has not yet been established by the balance of available evidence. Bevacizumab has shown efficacy as a first-line treatment in enhancing the PFS rate when given in combination with IFN-α, but its utility as monotherapy has not been demonstrated [27]. Presently, temsirolimus is the only targeted therapy shown to have an OS rate significantly greater than IFN-α alone [28]. For this reason, an ongoing Phase III trial of bevacizumab in combination with temsirolimus is underway to evaluate the potential synergistic benefit of the two agents in combinational therapy. Further research is needed to optimize

Table 1. Representative Phase III randomized trials of targeted therapy for stage IV renal cell carcinoma.

Author (year)	Patient sample size (n)	Treatment arm(s)	Dose and route of administration	TTP	OS	Ref.
Motzer et al. (2007)	750	Sunitinib vs IFN-α	Sunitinib: 50 mg p.o. for 4 weeks, then 2 weeks off IFN-α: 9 MU sc. three-times weekly	11 vs 5 months (p < 0.001)	Not reached	[24]
Escudier et al. (2007)	903	Sorafenib vs placebo	Sorafenib: 400 mg p.o. twice daily	5.5 vs 2.8 months (p < 0.01)	19.3 vs 15.9 months (p = 0.02)	[25]
Sternberg et al. (2009)	435	Pazopanib vs placebo	Pazopanib: 800 mg p.o. once daily	9.2 vs 4.2 months (p < 0.0001)	Not reached	[26]
Escudier et al. (2007)	649	Bevacizumab with IFN-α vs IFN-α with placebo	Bevacizumab: 10 mg/kg iv. daily IFN-α: 9 MU sc. three-times weekly	10.2 vs 5.4 months (p = 0.0001)	Not reached	[27]
Hudes et al. (2007)	626	Temsirolimus alone (n = 209) or temsirolimus with IFN-α (n = 210) vs IFN-α alone (n = 207)	Temsirolimus alone: 25 mg iv. weekly Temsirolimus with IFN-α: 15 mg iv. weekly with 3–6 MU sc. three-times weekly IFN-α: 3–9 MU sc. three-times weekly	Temsirolimus alone vs IFN-α alone: 5.5 vs 3.1 months Temsirolimus with IFN-α vs IFN-α alone: 4.7 vs 3.1 months	Temsirolimus alone vs IFN-α alone: 10.9 vs 7.3 months Temsirolimus with IFN-α vs IFN-α alone: 8.4 vs 7.3 months	[28]
Motzer et al. (2008)	416	Everolimus vs placebo	Everolimus: 5 mg p.o. twice daily	4.6 months vs 1.8 months (p < 0.0001)	Not reached	[29]
Amato et al. (2010)	733	TroVax® vs placebo with concurrent low-dose IL-2, IFN-α or sunitinib	TroVax: 1 × 10^9 TCID$_{50}$/ml im.	N/A	20.1 vs 19.2 months (p = 0.55)	[75]

im.: Intramuscular; iv.: Intravenous; MU: Million units; N/A: Not applicable; OS: Overall survival; p.o.: Per orem; sc.: Subcutaneous; TTP: Time to progression.

the effectiveness of approved targeted therapies in combination with cytokines and other novel immunotherapeutic moieties.

Rationale for TroVax® vaccine development

The concept that the immune system is capable of producing an antitumor response was first proposed over 100 years ago [30]. Through recent advances in molecular biology, immunology and cancer genetics, the molecular and cellular basis for tumor recognition and eradication by the immune system is becoming better understood. A complex feedback system exists between tumors and the host immune system. In the 1950s, Burnet and Thomas postulated that lymphocytes in circulation sought out malignant cells to enable their removal in a process known as immunosurveillance [31]. This paradigm has since been well established in murine models using conditional knockouts. An updated theory of immunosurveillance proposed by Schreiber and Old has been incorporated into a so-called 'immunoediting' model in which tumor cells expressing highly

immunogenic antigens are recognized by the innate immune system and lysed during an 'elimination' stage [32]. Release of intracellular contents into circulation enhances the activity of the adaptive arm of the immune system; this results in further tumor cell killing and the inadvertent selection of nonimmunogenic tumor cell variants. Tumor cell evasion from immune effector cells may occur through several proposed mechanisms, including antigen loss and MHC class I downregulation by tumor cells [33,34], potentiation of immunosuppression through selective cytokine release [35] and increased local and systemic proliferation of Tregs [36]. Over time, a stage of 'equilibrium' is reached when tumor eradication is balanced by tumor growth. When the majority of tumor cell variants can no longer be recognized by the immune system, tumor 'escape' occurs and cancer becomes clinically apparent.

Effective antitumor immunity requires recognition of transformed tumor cells by the immune system. Studies in T-cell and IFN-γ knockout mice suggested that tumor immunosurveillance is dependent on effector T cells.

This has implications for tumor immuno-therapy and, hence, mobilization of an effector T-cell population has been the primary goal for tumor immunotherapy [37]. Specifically, the role of CD8⁻ cytotoxic T lymphocytes (CTLs) has received the most attention because recognition of MHC class I-restricted epitopes by CTLs is expected to result in direct lysis of tumor cells. Recent evidence, however, has also highlighted the importance of CD4⁻ T helper cells in enhancing antitumor immunity. CD4⁻ T helper cells can be classified into subset populations based on their cytokine expression patterns and function [38]. Th1 cells generally produce IL-2 and IFN-γ and help generate CD8⁻ T-cell responses whereas Th2 cells produce IL-4, IL-5, IL-10 and help generate B-cell responses and antibody production. Another recently described CD4⁻ T-cell subset is the Th17 cell which produces IL-17 and mediates dendritic cell recruitment and release of inflammatory mediators within the tumor microenvironment [39]. While the specific roles of these cells in tumor immunity is not fully elucidated, several mechanisms of tumor escape have been well described. Tumor cells that downregulate MHC class I or tumor antigen expression may naturally escape CTL recognition and the absence of costimulation may induce T-cell tolerance. Local production of suppressive cytokines, such as IL-10, TGF-β and VEGF, also help promote peripheral T-cell tolerance.

B cells define the humoral arm of the adaptive immune system and may also act as antigen presenting cells (APCs). Upon terminal differentiation into plasma cells, they are capable of secreting antibodies which then potentiate target cell removal through a variety of means, including opsonization, activation of the complement system and by facilitating antibody-dependent cellular cytotoxicity by NK cells. However, while cancer patients very often develop high antibody titers against tumor-associated antigens (TAAs), this phenomenon has not been heretofore correlated with active protection against cancer. Moreover, recent studies have shown that B cells may depress CTL function through CD40-dependent inhibition of Th1 responses [40,41]. Alternatively, B cells may enhance antitumor immunity by acting as APCs or by providing costimulatory signals to T cells [42].

Tregs are a population of immunosuppressive lymphocytes which normally serve to maintain peripheral tolerance and prevent the development of autoimmune disease in healthy individuals. For immunotherapy, however, they represent a theoretical barrier that must be overcome in order to stimulate effective immune effector responses against tumors. Tregs are a subset of CD4⁻ T cells which express CD25 (IL-2Ra, the α-subunit of the IL-2 receptor) and which express high levels of the transcription factor FoxP3 [43]. IL-2 appears to play a critical role in mediating the suppressive function of Tregs. In a murine model, adoptive cell transfer of self-/tumor-specific CTLs into wild-type mice resulted in absence of tumor killing, whereas in IL-2Ra-deficient mice robust tumor killing activity was observed [44]. A recent Phase II, single-site clinical trial of 16 patients with metastatic melanoma receiving recombinant IL-2/diphtheria toxin conjugate (DAB/IL2) demonstrated partial to near-complete regression of metastatic lesions in five individuals. DAB/IL2 produced a transient depletion of Treg and T-effector cell populations, and upon T-cell repopulation several patients had developed melanoma antigen-specific CD8⁻ T-cell populations [45]. In another study, administration of high-dose IL-2 to patients with metastatic melanoma or mRCC resulted in decreased Treg populations in a subset of patients who were observed to have objective clinical responses to therapy [46]. As the current body of evidence suggests, removal of these immunosuppressive Tregs *in vivo* might be expected to enhance the overall potency of immune-based therapies for cancer.

Table 2. Defined antigens under investigation in renal cell carcinoma vaccine development.

Tumor antigen	Type	Expression pattern in renal cell carcinoma	Reported MHC-I restricted epitopes	Reported MHC-II restricted epitopes	Ref.
EphA2	Receptor tyrosine kinase	High	HLA-A2	HLA-DR4	[48]
5T4	Oncofetal	High (clear and papillary cell)	HLA-A1, -A2, -A3, -cw7, -B7	HLA-DR4	[49,51]
RAGE-1	Cancer-testis	Low (<2%)	Various	Various	[52]
NY-ESO-1	Cancer-testis	Low	HLA-A2, -A31	HLA-DP4	[53]
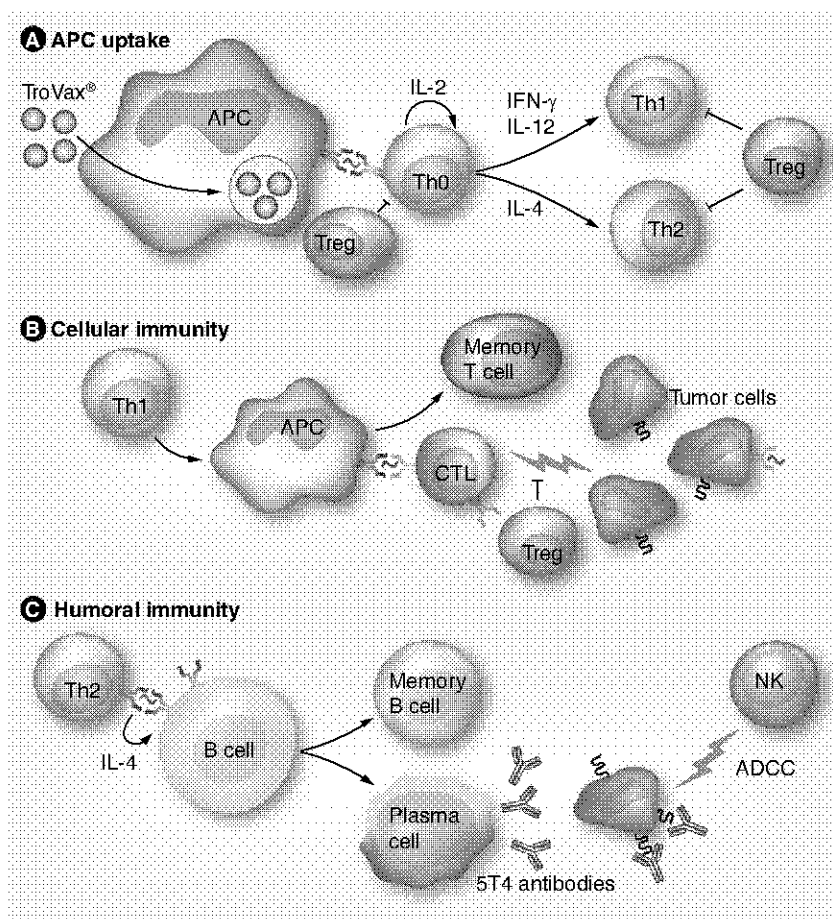
Figure 1. Potential mechanisms of anti-immunity induced by MVA-5T4 (TroVax®). (A) TroVax (spheres) is endocytosed by professional APCs, processed, and 5T4 epitopes are presented or cross-presented as peptides bound to MHC class II and I molecules, respectively. The MHC–peptide complex triggers recognition by cognate 5T4-specific T-cell receptors and induces maturation of CD4⁺ Th1 and/or Th2 helper T cells based on the selective cytokine expression profile. **(B)** Th1 cells enhance antigen presentation by the APCs to CD8⁺ effector T cells, which results in cytotoxic killing of target cells via granzyme/perforin release upon recognition of 5T4 on tumor cells. Memory T cells are also produced. **(C)** B cells are activated by APCs and supported by generation of Th2 CD4⁺ T cells. B cells differentiate into plasma cells and memory B cells. Plasma cells generate 5T4-specific antibodies, promoting ADCC by NK cells against the tumor. Regulatory T cells are thought to suppress the immune response by inhibiting maturation of APCs and T cells, and by blocking CD8⁺ T-cell effector function.
ADCC: Antibody-dependent cellular cytotoxicity; APC: Antigen-presenting cell; CTL: Cytotoxic T lymphocyte.

The existence of TAAs was initially demonstrated by murine studies in which proliferation of reinnoculated tumor cells was inhibited in mice immunized against both chemically and virally induced tumors [47]. An ideal TAA suitable for cancer vaccine development should bear the following characteristics: potent immunogenicity; constitutively present in tumor cells but not to any appreciable extent in normal cells; and having an inherent function that is linked to tumor progression or survival. While numerous antigens have been characterized across a range of tumor types, RCC has had relatively

few defined TAAs. Table 2 lists the characteristics of several defined antigens in RCC [48–53].

The cell surface antigen 5T4 is a 72 kDa N-glycosylated transmembrane protein initially discovered on placental trophoblasts but not found on adult human and murine cells [50,51]. 5T4 is not cleaved from the cell surface and therefore is not found in the peripheral circulation; this antigenic property is expected to enhance both antibody-dependent cellular cytotoxicity against the tumor cell and the generation of T-cell epitopes via intracellular degradation of full-length 5T4 [13]. 5T4 has been identified in various solid tumors including gastric, ovarian, colorectal and clear-cell and papillary RCC [54]. Along with its restricted expression profile, it is suitable as a target for vaccine therapy given its apparent function in mediating tumor adhesion and metastasis [55]; indeed, a high level of 5T4 expression is associated with a poor prognosis in metastatic colorectal, gastric, ovarian and non-small-cell lung adenocarcinoma [56]. Moreover, cells transfected with cDNA encoding 5T4 demonstrated increased motility and altered cell division behavior, further supporting its role in tumor invasion and progression [54]. In studies of healthy human subjects, clonal expansion of 5T4-specific CD4+ and CD8⁻ T-cell populations were induced via cross-priming with autologous dendritic cells transfected with a viral vector containing the 5T4 antigen [57,58].

Modified vaccinia Ankara (MVA) is a highly attenuated, replication-defective strain of the vaccinia virus developed by multiple passages of vaccinia virus in tissue culture [13]. MVA has undergone extensive testing in a large-scale smallpox vaccination study of 120,000 individuals; among individuals who were at high risk for developing vaccinia-related complications, adverse events related to MVA were not observed [59]. Moreover, owing to its large dsDNA genome, which ranks largest among mammalian viruses, vaccinia has proven to be a suitable vector for expressing large eukaryotic transgenes within a single viral vector [60]. MVA is additionally capable of enhancing the immunogenicity of its transgenes by virtue of the inflammatory nature of the poxvirus proteins [61]. These qualities make MVA an ideal vehicle for vaccine delivery. TroVax was generated as an MVA vector engineered to express 5T4. Figure 1 shows the possible mechanisms by which TroVax might generate *in vivo* immune response against tumor cells.

Preclinical development of TroVax
The safety profile and therapeutic efficacy of TroVax have been validated in preclinical studies

utilizing murine models. In one such study, MVA vectors expressing both the human (MVA-h5T4) and the murine (MVA-m5T4) analogs of 5T4 were constructed and inoculated into mice to simulate pulmonary metastases [62]. No evidence of autoimmune toxicity was observed in the mice. Delayed or absent growth activity of a self-antigen tumor model was observed in mice immunized with MVA-m5T4 compared with control groups. Furthermore, inoculation with MVA-h5T4 resulted in regression of established h5T4 pulmonary nodules [62].

In another study, mice inoculated with varying doses of TroVax were observed to have a significant degree of antitumor protection that persisted for up to 6 months on subsequent challenge with a syngeneic tumor line CT26 expressing human 5T4. Quantitative antibody responses determined by ELISA assay positively correlated tumor protection with high 5T4 antibody titers. The investigators found that mice receiving the highest viral inoculate had developed the highest 5T4 antibody titers, and in turn were also found to have the best protection against tumor challenge. In the same study, mice depleted of CD4⁻ T cells demonstrated continued tumor growth following TroVax administration, while CD8⁻ T-cell-depleted mice did not demonstrate tumor suppression. On passive transfer of 5T4-specific antibodies to mice with pre-existing lung tumors, there was an overall reduction in tumor burden [63]. From the results of this study, the investigators concluded that the mechanism of tumor rejection following TroVax is CD4⁻ T-cell dependent and antibody-mediated in mice.

A 10 amino acid HLA-Cw7-restricted epitope of 5T4 had been initially identified in a study of healthy subjects randomly screened for CD8⁻ T-cell activity against 5T4. IFN-γ secretion by CD8⁻ T cells was detectable via ELISPOT assay when cultured with a 10-mer peptide fragment of 5T4. The study subsequently showed that priming of CD8⁻ T cells with dendritic cells presenting the 5T4 10-mer peptide enabled recognition of autologous target cells expressing endogenous 5T4 [64]. A later study also validated the existence of HLA-A2-restricted 5T4 epitopes that could be used to expand CTL *in vitro* [49]. Another study had successfully demonstrated clonal expansion of 5T4-specific CD4⁻ T cells following depletion of CD25⁻ Tregs. In this study, a 5T4-derived 15-mer peptide was recognized by the T-cell clones in an HLA-DR4-restricted manner [58]. Thus, the discovery of HLA-restricted 5T4 epitopes capable of inducing clonal expansion of CTL and T helper cell

populations has provided a sound theoretical basis for the use of TroVax in therapeutic cancer immunotherapy.

Early phase clinical data with TroVax

An open-label, Phase I/II clinical trial evaluating the safety of TroVax in patients with advanced colorectal cancer was completed in 2005 [65]. The study cohort consisted of 22 immunocompetent adult patients with late-stage colorectal cancer who had been stabilized on first-line chemotherapy; this inclusion criteria was chosen to reflect the minimal lead time needed for any patient to generate an immune response, a period not otherwise afforded to patients with rapidly progressive disease, and who would otherwise be candidates for initiation of second-line chemotherapy. Patients were placed into four groups and were given a series of three doses of TroVax via intramuscular (im.) injection at either the 1×, 5× or 10× doses (defined as 5×10^7 pfu, 1×10^8 pfu and 2.5×10^8 pfu, respectively) over an 8-week period. The fourth group of patients was given the vaccine via intradermal (id.) injection three-times in 8 weeks at the 2x dose. TroVax was well tolerated in all groups and no serious adverse events were reported; grade 1–2 erythema at the injection site in the id. group was the most frequently reported event.

The immune response to TroVax administration was gauged by measuring serum 5T4-specific antibody responses by ELISA assay and 5T4-specific CD4⁻ T-cell activity was assessed by T-cell proliferation assay. In total, 14 patients developed 5T4-specific antibody titers. The T-cell proliferation assay demonstrated 5T4-specific proliferative activity in eight patients following two administrations of TroVax. A dose escalation effect was not observed among the 1×, 5× and 10× doses, nor was an improved immune response seen in the id. administration cohort. However, earlier induction of 5T4-specific T-cell proliferation activity was observed in the 10× dose group. Therefore, the optimal dosing and administration route selected for later studies was the 10× im. dose. On retrospective analysis of the study data, an increase in the 5T4-specific antibody response was correlated with increased time to progression and overall patient survival [65].

Subsequent Phase II studies of TroVax in colorectal cancer patients were conducted following the establishment of Phase I safety data [66–68]. Two open-label Phase II studies evaluated TroVax in combination with cytotoxic chemotherapy on the premise that chemotherapy might bolster the

efficacy of TroVax through a variety of mechanisms, including increased antigen cross-presentation, elimination of immunosuppressive cells and nonspecific activation of APCs [69]. TroVax was administered before, during, and after treatment with 5-fluorouracil, leucovorin and oxaliplatin (FOLFOX regimen) [67] or 5-fluorouracil, leucovorin and irinotecan (FOLFIRI regimen) [68]. No adverse events attributable to TroVax were observed in either study. 5T4-specific humoral and cellular responses were induced post-treatment in all evaluable patients in both studies. The studies found that TroVax/FOLFOX induced complete or partial response in six of 11 patients (54.5%), while TroVax/FOLFIRI induced complete or partial response in seven of 19 patients (36.8%), with an additional five patients achieving stable disease. Overall, the studies supported the use of TroVax as an adjuvant treatment to chemotherapy-responsive colorectal cancer.

TroVax was evaluated in men with hormone-refractory prostate cancer. In an open-label Phase II clinical trial of 27 patients who had failed androgen deprivation and chemotherapy regimens'. TroVax at the 10× dose was administered via the im. route alone or in combination with adjuvant GM-CSF given as 250 µg/m² by subcutaneous (sc.) injection [70]. A single dose of TroVax was administered on days 2, 13, 30, 41 and 58, with three booster injections being given every 28 days until week 21, then every 56 days until the 45th week. GM-CSF was given every 14 days in a 28-day cycle for a year's duration. No serious adverse outcomes attributable to TroVax were reported, and all 24 evaluable patients in the study developed 5T4-specific antibody responses; there was no appreciable difference in the rate of humoral responses between the monotherapy versus combined TroVax/GM-CSF arms following the second vaccination. Cellular response was determined by IFN-γ ELISPOT assay, wherein 38% of patients had positive reactions to the control peptide pool postvaccination. Retrospective analysis showed a significant improvement in the time to progression in patients with documented cellular responses. The addition of adjuvant GM-CSF did not significantly enhance immunological responses [70].

Clinical trials of TroVax in RCC

A total of five trials have been completed to date evaluating the safety and efficacy of TroVax in patients with mRCC. These include one Phase I/II trial and three Phase II trials which weighed the benefits of TroVax in combination with cytokine therapy. A large multi-institutional, randomized prospective Phase III trial of 733 patients has also been completed (see Table 3 for a summary of these clinical trials). As with the aforementioned studies in colorectal and prostate cancer, TroVax was well tolerated, with few serious adverse events being reported throughout the studies.

TroVax in combination with low-dose IL-2 was evaluated in a Phase II study of 25 patients with mRCC [71]. All patients received TroVax at the 10× im. dose and then IL-2. Each 5-day IL-2 cycle was repeated every 8 weeks; cycle one consisted of 250,000 U/kg/day sc. injection followed by 125,000 U/kg/day in subsequent cycles. A total of six cycles was completed with up to three additional cycles being given if the patient showed an objective response as determined by standard Response Evaluation Criteria in Solid Tumor (RECIST) guidelines. There were no vaccine-related adverse events reported in this study. A total of 21 patients developed 5T4-specific antibody titers following one or more vaccinations with TroVax. Five of 11 patients evaluable for cellular response were positive for 5T4-specific T-cell activity via IFN-γ ELISPOT assay. A complete clinical response which exceeded 24 months was observed in two patients, and a partial response which exceeded 12 months was seen in one patient. Of note, these three patients had above-the-median 5T4-specific antibody responses following three vaccinations. The median PFS for all patients was 3.37 months (range: 1.50–24.76 months), and the OS was 12.87 months (range: 1.90–24.76 months). A retrospective analysis of the data found that patients with above-the-median 5T4-specific antibody titers had significantly increased PFS (p = 0.02) and OS (p = 0.04).

A subsequent study of TroVax administered with high-dose IL-2 was evaluated in a Phase II, open-label trial [72]. To evaluate the potential synergy of TroVax with high-dose IL-2, 25 patients with advanced RCC were given TroVax at the 10× im. dose every 3 weeks. A 5-day cycle of 600,000 U/kg/day IL-2 was started after the second and third TroVax immunization, and was continued for one additional cycle in responding patients. No serious adverse events attributed to TroVax were seen in this study, and expected side effects related to high-dose IL-2 administration were reported. All 25 patients developed 5T4-specific antibodies by ELISA assay. Of the 23 patients with evaluable cellular responses, 13 (57%) showed 5T4-specific CD8⁻ T-cell responses measured by IFN-γ ELISPOT assay. Clinical responses determined by standard RECIST criteria were not observed in this study;

however, three patients were rendered disease-free following surgical resection. Stable disease was seen in 12 patients and was associated with an increased 5T4-specific CD8- T-cell response (p = 0.015). Moreover, patients with progressive disease exhibited post-treatment increases of CD4-CD25-FoxP3- Tregs, while patients with stable disease demonstrated decreased Treg levels.

Two clinical trials have been completed to evaluate TroVax in combination with IFN-α administration. The first of these clinical trials was a Phase II study of 28 patients with advanced RCC who were treated with TroVax monotherapy (n = 13) or in combination with IFN-α (n = 15) [73]. TroVax was given to all patients at the 10× im. dose on weeks 1, 3, 6, 9, 17, 28, 33 and 41. IFN-α was given by sc. administration to the combination group at the 6 million U/day three-times weekly for first week, followed by 9 million U/day three-times weekly for the subsequent weeks. No serious adverse events attributable to TroVax were reported in this study. Humoral and cellular immune response were evaluable in 23 of the study participants, of whom 21 (84%) demonstrated 5T4-specific antibody responses post-treatment, and 7 (33%) demonstrated 5T4-specific T-cell responses by ELISPOT assay. Patients in the TroVax plus IFN-α arm tended to have higher 5T4-specific antibody responses, while those in the TroVax alone arm tended to develop higher 5T4-specific cellular responses. Clinically, the addition of IFN-α was not associated with significant benefits in PFS or OS.

The second study evaluating TroVax in combination with IFN-α was a smaller Phase I/II open-label trial which enrolled 11 patients with advanced RCC [74]. The aim of the study was to assess the safety and immunogenicity of TroVax when administered in combination with IFN-α. Patients received TroVax at the 10× im. dose on weeks 1, 3, 6, 9, 17, 28, 33 and 41. IFN-α was administered at the 3 million U/day sc. three-times weekly dose for the first week, and then at the 9 million U/day sc. dose for subsequent weeks. No serious adverse events attributable to TroVax were reported. Of the 11 study participants, all mounted 5T4-specific antibody responses, and five (45%) exhibited 5T4-specific T-cell responses. Objective response as determined by RECIST criteria were not seen in the course of this study, although the median time to progression was noted to be 9 months for papillary RCC patients (range: 2.1–26+ months) and 10.4 months for clear cell patients (range: 3.9–26+ months), in both cases longer compared with IFN-α monotherapy.

Study/Phase	Patient sample size (n)	Treatment arm(s)	Number of TroVax injections (dose)	Clinical response CR (%)	PR (%)	SD (%)	PD (%)	TTP	OS	Immunological response (%) 5T4 Ab	5T4 IFN-γ ELISPOT	Ref.
Amato et al. Phase II	25	Concurrent low-dose IL-2	8 (10× im.)	2/25 (8)	1/25 (4)	6/25 (24)	15/25 (60)	3.37 months	12.87 months	21/25 (84)	5/11 (45)	[71]
Kaufman et al. Phase II	25	Concurrent high-dose IL-2	8 (10× im.)	0/25 (0)	0/23 (0)	12/23 (52)	11/23 (48)	4.76 months	>28 months	23/23 (100)	13/23 (57)	[72]
Hawkins et al. Phase I/II	11	Concurrent IFN-α	11 (10× im.)	0/11 (0)	0/11 (0)	10/11 (91)	1/11 (9)	10.4 months	N/A	11/11 (100)	5/11 (45)	[74]
Amato et al. Phase II	28	TroVax alone vs TroVax with concurrent IFN-α	9 (10× im.)	0/25 (0)	1/25 (4)	14/25 (56)	10/25 (40)	3.73 vs 6.07 months p = 0.13	5.93 vs 18.27 months p = 0.02	21/25 (84)	7/21 (33)	[73]
Amato et al. Phase III (TRIST)	733	TroVax vs placebo with concurrent low-dose IL-2, IFN-α or sunitinib	13 (planned), 8 (median received) (1 × 10⁹ TCID₅₀/ml im.)	N/A	N/A	N/A	N/A	N/A	20.1 vs 19.2 months p = 0.55	56 vs 6%	N/A	[75]

CR: Complete response; im.: Intramuscular; N/A: Not applicable; OS: Overall survival; PD: Progressive disease; PR: Partial response; SD: Stable disease; TTP: Time to progression.

The completed Phase II trials of TroVax have heretofore confirmed the safety profile in RCC and demonstrated a trend towards improved clinical benefit in patients with mRCC. A significant survival benefit was particularly seen in patients who had received concomitant IL-2 administration. Furthermore, the majority of patients had developed 5T4-specific antibody titers post-treatment, and a subset of patients also developed 5T4-specific cellular responses. Overall, these findings supported further evaluation of TroVax for RCC in the Phase III setting.

A Phase III trial designated TRIST was initiated in 2006. TRIST was an international multicenter, randomized, prospective trial that evaluated the efficacy of TroVax in adult patients with good-to-intermediate prognosis mRCC receiving standard-of-care treatments [75]. A total of 733 patients with metastatic or locally advanced clear cell RCC were recruited for the study, and in a double-blind manner randomized 1:1 to TroVax or placebo combined with physician-assigned low-dose IL-2, IFN-α or sunitinib. Primary study end point was defined as OS. Secondary end points included PFS, objective response rate and safety. Patients randomized to the TroVax treatment arm received vaccinations at the 1×10^9 TCID$_{50}$/ml im. dose during weeks 1, 3, 6, 9, 13, 17, 21, 25, 33, 41, 49, 57 and 65. Placebo was administered in an identical manner. A total of 365 patients received TroVax and 368 received placebo. First-line standard-of-care treatment was determined by local practice guidelines (physician preference): a total of 170 patients received low-dose IL-2 at the 250,000 U/kg/day (upper limit 22 million U per dose) five-times weekly sc. dose during the first cycle, followed by 125,000 U/kg/day (upper limit 11 million units per dose) five-times weekly in succeeding cycles; 371 patients received IFN-α sc. three-times weekly with doses ranging from 9 to 18 million units per dose; and 184 patients received sunitinib 50 mg p.o. daily on a schedule of 4 weeks on and 2 weeks off. Overall, there were no serious adverse events reported in either the TroVax or placebo arms related to vaccination.

Following an interim review by the Data Safety Monitoring Board (DSMB) in July 2008, TRIST was terminated when it was determined that the primary end point of OS could not be reasonably met [75]. At the time of the study's termination, the median time on the study was 6 months, and study participants had received a median of eight TroVax/placebo injections in combination with low-dose IL-2 or IFN-α and seven injections in combination with sunitinib. Survival data was censored to March 2009 to enable a median

follow-up time of 12.9 months. The median OS for the TroVax arm was 20.1 months and for the placebo arm 19.2 months (HR: 1.07; 95% CI: 0.86–1.32; p = 0.55). Retrospective subset analysis of the data revealed a positive association between high 5T4-specific antibody titers and enhanced survival in the TroVax group compared with the placebo group. While the TRIST study was not powered to assess survival differences between treatment groups, it was found that patients with favorable prognoses (as determined by MSKCC risk grade) and treated with TroVax plus low-dose IL-2 had better outcomes than those who received placebo plus low-dose IL-2 (HR: 0.54; 95% CI: 0.30–0.98; p = 0.046). A similar effect could not be observed in patients with MSKCC intermediate prognosis scores, and these patients were found to additionally have an elevated incidence of thrombocytosis. On further exploratory analysis, lower baseline platelet levels were associated with higher 5T4-specific antibody titers and improved survival (p = 0.04) [76].

Biomarkers associated with TroVax response

Data gathered from the Phase I/II trials of TroVax in RCC established a strong association between 5T4-specific antibody response and enhanced survival. Although the TRIST study was terminated after it failed to reach the primary end point of improved OS, in a prospectively planned analysis of the data a positive correlation between high 5T4-specific antibody titers and OS was found. It was not known at the time of the study's implementation which pretreatment factors contributed to the clinical benefit seen in patients. While it may simply be that enrolling healthier patients with robust immune systems could lead to better responses to TroVax, the TRIST study did not identify a relationship between MSKCC good prognosis and increased 5T4-specific antibody titers. Predictive biomarkers would be of substantial benefit in patient selection and in monitoring objective responses to immunotherapy. Such biomarkers could include baseline patient factors as well as serum and genomic markers.

In a retrospective analysis of the TRIST study data, a predictive criteria designated the immune response surrogate (IRS) was constructed by means of evaluating patients' baseline hematological, immunological, demographic and other cancer-associated variables [77]. Only those individual variables which related significantly to eventual 5T4-specific quantitative antibody responses as determined by ELISA assay were retained in the model. The variables that reached statistical

significance included baseline 5T4 antibody level, hemoglobin level and hematocrit. While each of these variables was independently associated with clinical responses on univariate analysis, it was noted that the sign associated with hematocrit was negative in the IRS. This indicates that for a given level of hemoglobin, therapeutic response is negatively associated with hematocrit.

The IRS was constructed as a linear regression model of the above variables, and applied towards TRIST data. Based on hazard ratio analyses, the IRS was determined to be a strong prognostic factor for survival in TroVax-treated patients (p < 0.0001) and weakly prognostic in the placebo group (p = 0.05). Because of the observed correlation of the IRS with survival benefit in patients receiving the vaccine, the investigators noted that TroVax must exert a degree of therapeutic activity in patients with RCC; otherwise, no difference in hazard ratios between treatment and placebo groups would have been seen.

When the IRS was retrospectively applied to an independent dataset consisting of 108 patients extracted from the Phase I/II TroVax trials in renal, colorectal and prostate cancer, it was found to be positively associated with both quantitative 5T4 antibody response and OS [77]. It is also of note that this study demonstrated a lack of a significant correlation between quantitative anti-MVA antibody titers and survival, which supports the hypothesis that response to TroVax is not simply a consequence of better overall patient health. In comparison with MSKCC risk criteria, the IRS does appear to hold more prognostic value in predicting clinical responses specific to TroVax (see Figure 2). Similar trends between the patient IRS and clinical responses were seen in clinical trials of TroVax in colorectal and prostate cancer patients. There is a need to prospectively validate the utility of the IRS before it can gain widespread use in the evaluation of anticancer therapies in RCC and perhaps other tumors.

Assessment of the TRIST study

There are a number of explanations to explain why the TRIST study failed to demonstrate improved survival in RCC. First, the approach to patient selection may not have been optimized at the onset of the study, as no predictive biomarkers were in place to predict the degree of benefit that could be derived from TroVax therapy. Phase II studies had suggested that TroVax in combination with IL-2 might benefit patients with good baseline performance

parameters. This was partially supported by the retrospective subset analysis of TRIST data demonstrating improved survival in RCC patients treated with TroVax and low-dose IL-2. The observations gleaned from the unplanned subset analysis should be validated in a future prospective study.

The kinetics of antitumor responses with TroVax are not fully known at this time. If indeed early immunobiochemical changes and delayed clinical responses are features of active immunotherapy, then assessment of outcomes by RECIST criteria alone would not be sufficient in evaluating the therapeutic benefit of these treatment modalities [78]. In fact, the recently FDA-approved ipilimumab for metastatic melanoma had, in its Phase III trial design, eschewed reliance on conventional tumor response criteria to allow ipilimumab to achieve improved survival rates when measured at later time points [79]. Therefore, in future studies of TroVax, particular attention should be directed towards establishing end points that allow for sufficient time to pass before separation of Kaplan–Meier survival curves may occur [80].

A second underlying problem lies in the unclear mechanisms by which TroVax induces antitumor immunity. Across all studies, TroVax had been shown to induce 5T4-specific antibody titers, while cytotoxic CD8⁻ T-cell responses have been less consistently seen and were in many cases transient. A better understanding of whether the activity of TroVax favors the humoral or cellular arms of adaptive immunity would thus aid in the design of future trials. The presence of Tregs in the tumor microenvironment presents another barrier to therapeutic efficacy. The optimal dosing of IL-2 and its role in mediating the immunosuppressive activity of Tregs will require further study. Furthermore, given that the majority of enrolled patients in the TRIST study came from eastern European sites, it is not known what local treatment paradigms or epigenetic factors across different populations with RCC might have affected the response to TroVax therapy.

A third set of factors involves a lack of knowledge about the optimal dosing and scheduling of TroVax vaccinations and booster administrations. The 10× im. dose was derived from an early-phase study of TroVax in colorectal cancer and was neither specific nor optimized for use in RCC and the TRIST trial. Compared to colorectal cancer, RCC may require additional boosters of TroVax spaced closer together to

MSKCC criteria risk factors [8]	Risk stratification groups
Karnofsky performance status <80%	Good risk: no risk factors
Lactate dehydrogenase level >15% of upper limit of normal	Intermediate risk: one risk factor
	Poor risk: two or more risk factors
Serum hemoglobin < lower limit of normal	
Absence of prior nephrectomy	
IRS pretreatment variables [77]	**Direction of effect on outcome**
Baseline 5T4 antibody level	Negative
Baseline hematocrit	Positive
Baseline hemoglobin level	Positive

Figure 2. Comparison of Memorial Sloan–Kettering Cancer Center and immune response surrogate biomarkers.
IRS: Immune response surrogate; MSKCC: Memorial Sloan–Kettering Cancer Center.

produce a durable clinical response. Likewise, more booster administrations may be needed on an ongoing basis as a large number of patients throughout Phase II studies of RCC had developed stable disease. Again, this may also be suggestive of a delayed therapeutic response to TroVax. Finally, the concurrent administration of IL-2, IFN-α or sunitinib at variable doses was based on physician preference and did not derive from a standardized protocol. Although Phase II RCC studies have evaluated cytokine therapies with TroVax, no such studies have been conducted on the safety and efficacy of sunitinib with TroVax. This could have significantly altered outcome data.

Conclusion & future perspective

A possible direction for future studies involving TroVax might involve combination therapy with other immunomodulatory agents. The data generated thus far suggests a potential benefit of TroVax when administered with IL-2 [71–72,75]. While the potential clinical benefit was suggested from nonrandomized Phase II trial results and subset analysis of the Phase III trial, further prospective, randomized studies evaluating TroVax with high-dose IL-2 are justified. Another strategy would be to consider combinations with anti-CTLA-4 and/or anti-PD-1 monoclonal antibodies. Cytotoxic T-lymphocyte antigen-4 (CTLA-4) is an immunomodulatory molecule preferentially expressed by CD4⁻CD25⁻FoxP3⁻ Tregs and acts to suppress T-cell proliferation. Blockade of CTLA-4 has

been shown to interfere with Treg function [81]. PD-1 is a negative costimulatory molecule that both depresses T-cell effector and proliferative functions and promotes Treg expansion [82]. A recent study implementing the dual blockade of CTLA-4 and PD-1 demonstrated high rates of tumor-specific T-cell activity against melanoma lesions compared with blockade of either molecule alone [83]. Next, blockade of tumor-released factors including IL-10, VEGF and TGF-β may also increase vaccine efficacy via modulation of tumor escape mechanisms [84,85]. Finally, toll-like receptor agonists may further enhance T-cell and NK cell responses, enhancing the activity of both innate and adaptive arms of the immune system against the tumor [86].

A study has reported the presence of a novel subset of neoplastic cells expressing the mesenchymal stem cell marker CD105 in patients with RCC [87]. These progenitor cells are thought to initiate the tumorigenic process through the release of paracrine mediators termed microvesicles. In a recent murine study, investigators isolated microvesicles from CD105⁻ cell suspension samples and found that *in vivo* implantation into severe combined immunodeficient mice triggered angiogenic activation of previously implanted human epithelial cells [88]. Given the potential role of microvesicles in enhancing tumor vascularization and metastasis in RCC, it would be of value to study the interaction of TroVax in mediating the release and function of the microvesicles in the tumor microenvironment.

In conclusion, MVA-5T4 (TroVax) is a recombinant viral vector expressing the TAA 5T4, developed for the purpose of inducing active antitumor immunity. The safety of TroVax has been well documented in numerous preclinical murine models and subsequent clinical trials of patients with colorectal, renal and prostate cancers. Phase II trials of TroVax had demonstrated the induction of 5T4-specific antibody responses in the majority of patients, and in select trials this finding was correlated with enhanced survival. On the basis of promising Phase II trial results, a Phase III trial in RCC designated TRIST was carried out. Ultimately, TRIST was terminated when it was found that the primary efficacy end point of OS could not be reasonably met. To optimize future studies of TroVax in renal cell and other applicable cancers, a number of improvements could be envisioned. First, particular attention should be given towards better patient selection by utilizing predictive biomarkers such as the IRS prior to patient enrollment. Second, with respect to the delayed kinetics of immunotherapy, appropriate primary end points of efficacy should be developed and protocols for dosing and vaccination/boosting schedules should be optimized. Third, combinational therapies or concurrent administration of TroVax with immune adjuvants, including high- or low-dose IL-2, costimulatory molecules, and Toll-like receptor agonists should be evaluated in subsequent studies of TroVax. Finally, continued research into the immunosuppressive dynamics of the tumor microenvironment could provide new strategies in enhancing the therapeutic benefit of TroVax.

Executive summary

- Surgical resection is the mainstay of treatment for early renal cell carcinoma. Adjuvant therapy with radiation and chemotherapy are not associated with improved survival.
- IL-2 has been a standard of care for metastatic renal cell carcinoma (mRCC) and suggests that the immune system can be used to treat the disease.
- Targeted molecular therapies are also available for first- and second-line applications in mRCC.
- TroVax® (MVA-5T4) is a modified vaccinia virus Anakar encoding the 5T4 tumor-associated antigen, which is present on the surface of RCC cells.
- TroVax has been associated with protection against tumors by enhancing antibody and CD4+ T-cell functions of the immune system in murine models, although the mechanism of tumor rejection in cancer patients remains speculative.
- The safety profile and efficacy of TroVax has been validated in preclinical murine studies.
- HLA-restricted epitopes of 5T4 were isolated and could be used to expand 5T4-specific T cells in vivo. Depletion of Tregs enhanced this effect.
- Phase I and II studies with TroVax in colorectal and prostate cancer demonstrated that TroVax was well tolerated and had a promising efficacy profile.
- Five trials of TroVax in mRCC have been completed to date and TroVax was well tolerated across all studies.
- A clinical benefit was suggested in patients treated with combination TroVax and IL-2 therapy in two Phase II trials and in a subset of patients treated on a Phase III study.
- The Phase III, double-blinded trial of TroVax in 733 patients with mRCC (TRIST) was carried out to assess the efficacy of TroVax versus placebo when used in addition to low-dose IL-2, IFN-α or sunitinib selected by physician preference.
- TRIST was halted after the predefined primary efficacy end point of increased overall survival could not be reasonably met by the timeframe set by the study.
- An immune response surrogate was constructed via a retrospective analysis of the TRIST data. Baseline 5T4 antibody level, hemoglobin level and hemacrit comprise the immune response surrogate, which was shown to be highly predictive of therapeutic response across all TroVax clinical trials.
- The TRIST study might have benefited from better patient selection, optimization of dosing parameters, tighter control of concurrent standard-of-care treatments, and readjustment of primary end points to better reflect the novel kinetics of tumor immunotherapy.
- A subset of patients treated with TroVax and concurrent low-dose IL-2 were found to have improved survival. This observation warrants further study of this combination regimen or with other strategies that overcome local immunosuppression.

References

Papers of special note have been highlighted as:
* of interest

1   *American Cancer Society. Cancer Facts and Figures 2011.* Atlanta, GA, USA. American Cancer Society (2011).

2   Young R. Metastatic renal cell carcinoma: what causes occasional dramatic regressions? *N. Engl. J. Med.* 338, 1305–1306 (1998).

3   Motzer RJ, Bander NH, Nanus DM. Renal-cell carcinoma. *N. Engl. J. Med.* 335, 865–875 (1996).

4   Woldrich JM, Mallin K, Ritchey J, Carroll PR, Kane CJ. Sex differences in renal cell cancer presentation and survival: an analysis of the National Cancer Database, 1993–2004. *J. Urol.* 179(5), 1709–1713 (2008).

5   Kidney. In: *AJCC Cancer Staging Manual.* Edge SB, Byrd DR, Compton CC (Eds). Springer, NY, USA, 479 (2009).

6   Patard JJ, Leray E, Rioux-Leclercq N *et al.* Prognostic value of histologic subtypes in renal cell carcinoma: a multicenter experience. *J. Clin. Oncol.* 23(12), 2763–2771 (2005).

7   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.* 17(8), 2530–2540 (1999).

8   Suppiah R, Shaheen PE, Elson P *et al.* Thrombocytosis as a prognostic factor for survival in patients with metastatic renal cell carcinoma. *Cancer* 107(8), 1793–1800 (2006).

9   Flaherty KT, Fuchs CS, Colditz GA *et al.* A prospective study of body mass index, hypertension, and smoking and the risk of renal cell carcinoma (United States). *Cancer Causes Control* 16(9), 1099–1106 (2005).

10 Lonser RR, Glenn GM, Walther M *et al.* Von Hippel-Lindau disease. *Lancet* 361(9374), 2059–2067 (2003).

11 Hu J, Mao Y, White K. Renal cell carcinoma and occupational exposure to chemicals in Canada. *Occ. Med.* 52(3), 157–164 (2002).

12 *Cancer Treatment and Research: Kidney Cancer.* Figlin RA (Ed.). Volume 116 (2003).

13 Prasad SR, Humphrey PA, Catena JR *et al.* Common and uncommon histologic subtypes of renal cell carcinoma: imaging spectrum with pathologic correlation. *Radiographics* 26, 1795–1806 (2006).

14 Cheville JC, Lohse CM, Zincke H, Weaver AL, Blute ML. Comparisons of outcome and prognostic features among histologic subtypes of renal cell carcinoma. *Am. J. Surg. Pathol.* 27(5), 612–624 (2003).

15 Motzer RJ, Bacik J, Mariani T *et al.* Treatment outcome and survival associated with metastatic renal cell carcinoma of non clear-cell histology. *J. Clin. Oncol.* 20, 2376–2381 (2002).

16 Kane CJ, Mallin K, Ritchey J, Cooperberg MR, Carroll PR. Renal cell cancer stage migration: analysis of the National Cancer Center Database. *Cancer* 113(1), 78–83 (2008).

17 Resnick MI, Older RA. *Diagnosis of Genitourinary Disease (2nd Edition).* Thieme Medical Publishers, NY, USA, 168–170 (1997).

18 Motzer RJ, Russo P. System therapy for renal cell carcinoma. *J. Urol.* 163(2), 408–417 (2000).

19 Lerner SE, Hawkins CA, Blute ML *et al.* Disease outcome in patients with low stage renal cell carcinoma treated with nephron sparing or radical surgery. *J. Urol.* 155(6), 1868–1873 (1996).

20 Milowsky MI, Nanus DM. Chemotherapeutic strategies for renal cell carcinoma. *Urol. Clin. North Am.* 30(3), 601–609 (2003).

21 Clement JM, McDermott DF. The high-dose aldesleukin (IL-2) "select" trial: a trial to prospectively validate predictive models of response to high-dose IL-2 treatment in patients with metastatic renal cell carcinoma. *Clin. Genitourin. Cancer* 7(2), E7–E9 (2009).

22 Rosenberg SA, Yang JC, White DE, Steinberg SM. Durability of complete responses in patients with metastatic cancer treated with high-dose interleukin-2: identification of the antigens mediating response. *Ann. Surg.* 228(3), 307–319 (1998).

23 Gore ME, Griffin CL, Hancock B *et al.* Interferon alfa-2a versus combination therapy with interferon alfa-2a, interleukin-2, and fluorouracil in patients with untreated metastatic renal cell carcinoma (MRC RE04/ EORTC GU 30012): an open-label randomised trial. *Lancet* 375(9715), 641–648 (2010).
*   Recent clinical trial demonstrating significant survival benefit with immunotherapy in patients with renal cell carcinoma.

24 Motzer RJ, Hutson TE, Tomczak P *et al.* Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. *N. Engl. J. Med.* 356, 115–124 (2007).
*   Clinical trial establishing the benefit of targeted therapy with sunitinib in metastatic renal cell carcinoma.

25 Escudier B, Eisen T, Stadler WM *et al.* Sorafenib in advanced clear-cell renal-cell carcinoma. *N. Engl. J. Med.* 356, 125–134 (2007).
*   Clinical trial establishing the benefit of targeted therapy with sorafenib in metastatic renal cell carcinoma.

26 Sternberg CN, Szczylik C, Lee E *et al.* A randomized, double-blind Phase III study of pazopanib in treatment-naive and cytokine-pretreated patients with advanced renal cell carcinoma (RCC). *J. Clin. Oncol.* 27, 5021 (2009).
*   Clinical trial establishing the benefit of targeted therapy with pazopanib in metastatic renal cell carcinoma.

27 Escudier B, Pluzanska A, Koralewski P *et al.* Bevacizumab plus interferon alfa-2a for treatment of metastatic renal cell carcinoma: a randomised, double blind Phase III trial. *Lancet* 370, 2103–2111 (2007).
*   Clinical trial that established an improvement in progression-free survival for metastatic renal cell carcinoma patients treated with IFN-α-2a and bevacizumab.

28 Hudes G, Carducci M, Tomczak P *et al.* Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. *N. Engl. J. Med.* 356, 2271–2281 (2007).
*   Clinical trial establishing the benefit of targeted therapy with temsirolimus in metastatic renal cell carcinoma.

29 Motzer RJ, Escudier B, Oudard S *et al.* Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled Phase III trial. *Lancet* 372, 449–456 (2008).
*   Clinical trial establishing the benefit of targeted therapy with everolimus in metastatic renal cell carcinoma.

30 Coley WB. The treatment of malignant tumors by repeated inoculations of erysipelas: with a report of ten original cases. *Am. J. Med. Sci.* 105, 487–511 (1893).

31 Burnet FM. The concept of immunological surveillance. *Prog. Exp. Tumor Res.* 13, 1–27 (1970).

32 Kim R, Emi M, Tanabe K. Cancer immunoediting: from immune surveillance to immune escape. *Immunology* 121(1), 1–14 (2007).

33 Chang CC, Ogino T, Mullins DW *et al.* Defective human leukocyte antigen class I-associated antigen presentation caused by a novel beta2-microglobulin loss-of-function in melanoma cells. *J. Biol. Chem.* 281, 18763–18773 (2006).

34 Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science* 331(6024), 1565–1570 (2011).
*   Excellent review of tumor immunosurveillance.

35 Sharma S, Stolina M, Lin Y *et al.* T cell-derived IL-10 promotes lung cancer growth by suppressing both T cell and APC function. *J. Immunol.* 163, 5020–5028 (1999).

36 Elkord E, Dangoor A, Burt DJ *et al.* Immune evasion mechanisms in colorectal cancer liver metastasis patients vaccinated with TroVax (MVA-5T4). *Cancer Immunol. Immunother.* 58, 1567–1567 (2009).

37 Jager E, Jager D, Knuth A. Antigen-specific immunotherapy and cancer vaccines. *Int. J. Cancer* 106, 817–820 (2003).

38 Morel PA, Oriss TB. Crossregulation between Th1 and Th2 cells. *Crit. Rev. Immunol.* 18(4), 275–303 (1998).

39 Zou W, Restifo NP. T(H)17 cells in tumour immunity and immunotherapy. *Nat. Rev. Immunol.* 10(4), 248–256 (2010).

40 Qin Z, Richter G, Schuler T, Ibe S, Cao X, Blankenstein T. B cells inhibit induction of T cell-dependent tumor immunity. *Nat. Med.* 4, 627–630 (1998).

41 Shah S, Divekar AA, Hilchey SP *et al.* Increased rejection of primary tumors in mice lacking B cells: Inhibition of anti-tumor CTL and TH1 cytokine responses by B cells. *Int. J. Cancer* 117, 574–586 (2005).

42 DiLillo DJ, Yanaba K, Tedder TF. B cells are required for optimal CD4+ and CD8+ T cell tumor immunity: therapeutic B cell depletion enhances B16 melanoma growth in mice. *J. Immunol.* 184(7), 4006–4016 (2010).

43 Shevach EM. CD4+ CD25+ suppressor T cells: More questions than answers. *Nat. Rev. Immunol.* 2, 389–400 (2002).

44 Antony PA, Paulos CM, Ahmadzadeh M *et al.* Interleukin-2-dependent mechanisms of tolerance and immunity *in vivo*. *J. Immunol.* 176, 3301–3305 (2006).

45 Rasku MA, Clem AL, Telang S *et al.* Transient T cell depletion causes regression of melanoma metastases. *J. Transl. Med.* 6, 12 (2008).

46 Cesana GC, DeRaffele G, Cohen S *et al.* Characterization of CD4+CD25+ regulatory T cells in patients treated with high-dose interleukin-2 for metastatic melanoma or renal cell carcinoma. *J. Clin. Oncol.* 24, 1169–1177 (2006).

47 Prehn RT, Main JM. Immunity to methylcholanthrene-induced sarcomas. *J. Natl Cancer Inst.* 18, 769–778 (1957).

48 Tatsumi T, Herrem CJ, Olson WC *et al.* Disease stage variation in CD4+ and CD8+ T-cell reactivity to the receptor tyrosine kinase EphA2 in patients with renal cell carcinoma. *Cancer Res.* 63, 4481–4489 (2003).

49 Shingler WH, Chikoti P, Kingsman SM, Harrop R. Identification and functional validation of MHC class I epitopes in the tumor-associated antigen 5T4. *Int. J. Immunol.* 20, 1057–1066 (2008).

50 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* 61(1), 89–95 (1990).

51 Hole N, Stern PL. A 72 kD trophoblast glycoprotein defined by a monoclonal antibody. *Br. J. Cancer* 57, 239–246 (1998).

52 Neumann E, Engelsberg A, Decker J *et al.* Heterogeneous expression of the tumor-associated antigens RAGE-1, PRAME, and glycoprotein 75 in human renal cell carcinoma: candidates for T-cell-based immunotherapies? *Cancer Res.* 58(18), 4090–4095 (1998).

53 Nicholau T, Ebert L, Davis ID *et al.* Directions in the immune targeting of cancer: lessons learned from the cancer-testis Ag NY-ESO-1. *Immunol. Cell Biol.* 84, 303–317 (2006).

54 Woods AM, Wang WW, Shaw DM *et al.* Characterization of the murine 5T4 oncofoetal antigen: a target for immunotherapy in cancer. *Biochem. J.* 366, 353–365 (2002).

55 Carsberg CJ, Myers KA, Evans GS, Allen TD, Stern PL. Metastasis-associated 5T4 oncofoetal antigen is concentrated at microvillus projections of the plasma membrane. *J. Cell. Sci.* 108, 2905–2916 (1995).

56 Naganuma H, Kono K, Mori Y *et al.* Oncofetal antigen 5T4 expression as a prognostic factor in patients with gastric cancer. *Anticancer Res.* 22, 1033–1038 (2002).

57 Smyth LJC, Elkord E, Taher TE *et al.* CD8 T-cell recognition of human 5T4 oncofetal antigen. *Int. J. Cancer* 119(7), 1638–1647 (2006).

58 Elkord E, Burt DJ, Drijfhout JW, Hawkins RE, Stern PL. CD4+ T-cell recognition of human 5T4 oncofetal antigen: implications for initial depletion of CD25+ T cells. *Cancer Immunol. Imunother.* 57, 833–847 (2008).

59 Stickl H, Hochstein-Mintzel V, Mayr A, Huber HC, Schäfer H, Holzner A. MVA vaccination against smallpox: clinical tests with an attenuated live vaccinia virus strain. *Dtsch Med. Wochenschr.* 99(47), 2386–2392 (1974).

60 Arlen PM, Kaufman HL, DiPaola RS. Pox viral vaccine approaches. *Semin. Oncol.* 32, 549–555 (2005).

61 Moroziewicz D, Kaufman HL. Gene therapy with poxvirus vectors. *Curr. Opin. Mol. Ther.* 7, 317–325 (2005).

62 Mulryan K. Ryan MG, Myers KA *et al.* Attenuated recombinant vaccinia virus expressing oncofetal antigen (tumor-associated antigen) 5T4 induces active therapy of established tumors. *Mol. Cancer Ther.* 1, 1129–1137 (2002).

63 Harrop R, Ryan MG, Myers KA, Redchenko I, Kingsman SM, Carroll MW. Active treatment of murine tumors with a highly attenuated vaccinia virus expressing the tumor associated antigen 5T4 (TroVax) is CD4+ T cell dependent and antibody mediated. *Cancer Immunol. Immunother.* 55(9), 1081–1090 (2006).

64 Redchenko I, Harrop R, Ryan MG, Hawkins RE, Carroll MW. Identification of a major histocompatibility complex class I-restricted T-cell epitope in the tumour-associated antigen, 5T4. *Immunology* 118(1), 50–57 (2006).

65 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.* 12, 3416–3424 (2006).

• First clinical trial of TroVax® in cancer patients.

66 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.* 31, 820–829 (2008).

67 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.* 13, 4487–4494 (2007).

68 Harrop R, Drury N, Shingler W *et al.* Vaccination of colorectal cancer patients with TroVax given alongside chemotherapy (5-fluorouracil, leucovorin, and irinotecan) is safe and induces potent immune responses. *Cancer Immunol. Immunother.* 57, 977–986 (2008).

69 Ramakrishnan R, Antonia S, Gabrilovich DI. Combined modality immunotherapy and chemotherapy: a new perspective. *Cancer Immunol. Immunother.* 57, 1523–1529 (2008).

70 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.* 31, 577–585 (2008).

71 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.* 14, 7504–7510 (2008).

Phase II clinical trial demonstrating clinical efficacy of TroVax administered with low-dose IL-2 in patients with metastatic renal cell carcinoma.

72 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.* 7, 2 (2009).

Phase II clinical trial demonstrating clinical efficacy of TroVax administered with high-dose IL-2 in patients with metastatic renal cell carcinoma.

73 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-alpha (IFNalpha): a Phase 2 trial. *J. Immunother.* 32, 765–772 (2009).

74 Hawkins RE, McDermott C, Shablak A *et al.* Vaccination of patients with metastatic renal cell cancer with modified vaccinia Ankara encoding the tumor antigen 5T4 (TroVax) given alongside interferon-alpha. *J. Immunother.* 32, 424–429 (2009).

75 Amato RJ, Hawkins RE, Kaufman HL *et al.* Vaccination of metastatic renal cancer patients with MVA-5T4: a randomized, double-blind, placebo-controlled Phase III study. *Clin. Cancer Res.* 16(22), 5539–5547 (2010).

Phase III international, prospective, randomized clinical trial of TroVax in patients with metastatic renal cell carcinoma.

76 Kim DW, Krishnamurthy V, Bines SD, Kaufman HL. TroVax: a recombinant modified vaccinia ankara virus encoding 5T4: lessons learned and future development. *Hum. Vacc.* 6(10), 1–8 (2011).

77 Harrop R, Shingler WH, McDonald M *et al.* MVA–5T4-induced immune responses are an early marker of efficacy in renal cancer patients. *Cancer Immunol. Imunother.* 60(6), 829–837 (2011).

Retrospective review establishing an immune response surrogate for predicting clinical responses to TroVax.

78 Wolchok JD, Hoos A, O'Day S *et al.* Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. *Clin. Cancer Res.* 15(23), 7412–7420 (2009).

Review of new clinical response guidelines for evaluating patients on clinical tumor immunotherapy trials.

79 Hodi FS, O'Day SJ, McDermott DF *et al.* Improved survival with ipilimumab in patients with metastatic melanoma. *N. Engl. J. Med.* 363(8), 711–723 (2010).

80 Hoos A, Eggermont AM, Janetzki S *et al.* Improved endpoints for cancer immunotherapy trials. *J. Natl Cancer Inst.* 102(18), 1388–1397 (2010).

81 Jain N, Nguyen H, Chambers C, Kang J. Dual function of CTLA-4 in regulatory T cells and conventional T cells to prevent multiorgan autoimmunity. *Proc. Natl Acad. Sci. USA* 107(4), 1524–1528 (2010).

82 Francisco LM, Sage PT, Sharpe AH. The PD-1 pathway in tolerance and autoimmunity. *Immunol. Rev.* 236(1), 219–242 (2010).

83 Curran MA, Montalvo W, Yagita H, Allison JP. PD-1 and CTLA-4 combination blockade expands infiltrating T cells and reduces regulatory T and myeloid cells within B16 melanoma tumors. *Proc. Natl Acad. Sci. USA* 107(9), 4275–4280 (2010).

84 Finke JH, Rini B, Ireland J *et al.* Sunitinib reverses type-1 immune suppression and decreases T-regulatory cells in renal cell carcinoma patients. *Clin. Cancer Res.* 14(20), 6674–6682 (2008).

85 Takaku S, Terabe M, Ambrosino E *et al.* Blockade of TGF-beta enhances tumor vaccine efficacy mediated by CD8+ T cells. *Int. J. Cancer* 126(7), 1666–1674 (2010).

86 Huang B, Zhao J, Li H *et al.* Toll-like receptors on tumor cells facilitate evasion of immune surveillance. *Cancer Res.* 65, 5009–5014 (2005).

87 Bussolati B, Bruno S, Grange C, Ferrando U, Camussi G. Identification of a tumor-initiating stem cell population in human renal carcinomas. *FASEB J.* 22, 3696–3705 (2008).

88 Grange C, Tapparo M, Collino F *et al.* Microvesicles released from human renal cancer stem cells stimulate angiogenesis and formation of lung premetastatic niche. *Cancer Res.* 71, 5346–5356 (2011).