Kidney Cancer

Mode-of-Action, Efficacy, and Safety of a Homologous Multi-Epitope Vaccine in a Murine Model for Adjuvant Treatment of Renal Cell Carcinoma


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Abstract

Background and objective: In a phase-III trial it was recently shown that an adjuvant renal cell carcinoma (RCC) vaccine (Reniale) reduces the risk of tumour progression following nephrectomy. This clinical trial focused on efficacy and did not investigate end-points relating to mode-of-action of the vaccine. In a murine model we investigated mode-of-action, efficacy, and safety of a homologous RENCA cell-based vaccine.

Design, setting, and participants: Six groups with 12 BALB/c mice per group received five vaccinations (lysate of 1 x 10^6–1 x 10^7 RENCA cells, manufactured with or without prior IFN-γ incubation) at 3-wk intervals before tumour transplantation and one vaccination 14 d afterwards. Controls (12 mice) received only solvent. All mice were sacrificed 21 d after tumour transplantation.

Measurements: Animal welfare, tumour growth, number of metastases, and the presence of cytotoxic T-lymphocytes as determined by a 51chromium-release assay. Adoptive immune transfer experiments (vaccination of nine mice with the RENCA vaccine or saline and transfer of serum, spleen cells, and CD4 and/or CD8 depleted spleen cells into five recipient mice each) were carried out to demonstrate involvement of different immune mechanisms.

Results: All controls developed a renal tumour, compared to 7/72 animals (9.7%) in the vaccine groups. The mean number of lung metastases was 100 (range 3–750) in controls and 4 (range 0–196) in the vaccine groups, respectively. Tumour uptake and number of metastases were not related to the vaccine dose. The 51chromium-release assay confirmed a significant tumour-specific cytolytic activity and marginally increased NK activity of splenocytes from vaccinated mice against RENCA cells compared to controls. Adoptive immune transfer experiments showed that the antitumoural effective immune mechanisms are cell-based.

Conclusions: We could demonstrate the mode-of-action, efficacy, and safety of a homologous tumour vaccine in a RENCA model. These findings support the positive results from a phase-III trial with Reniale.

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1. **Introduction**

Almost 3% of all malignant tumours in adults are renal cell carcinomas (RCC). In 2008, 54,390 new cases and 13,010 deaths of RCC and renal pelvis cancer are expected in the United States [1]. The overall 5-yr relative survival rate of all patients with RCC is 65% [2]. Standard therapy of organ-confined or regionally advanced RCC is partial or radical nephrectomy. RCCs are localized in 55%, regionally advanced in 19%, and distant in 20% of patients with corresponding 5-yr survival rates of 90%, 62%, and 10%, respectively [2]. Despite this significant stage-related risk of tumour progression, no effective adjuvant treatment following radical nephrectomy could be established in the past [3–7].

Recently, we have shown in a randomized phase-III trial that an adjuvant RCC vaccine (Reniale) is effective in reducing the risk of tumour progression in patients with RCC following radical nephrectomy [8]. This clinical phase-III trial focused on efficacy and did not investigate end-points relating to the mode-of-action of the vaccine. In a series of nonclinical experiments, we investigated the mode-of-action of a homologous RENCA cell-based vaccine in a murine model that mimics the human situation as closely as possible. The RENCA model fulfills several requirements for a useful animal model: the tumour cells are of spontaneous origin, are syngeneic to BALB/c mice and confirmed to be an adenocarcinoma, have a predictable growth rate both in vitro and in vivo, and form a primary mass and metastases in the mice [9–12].

In this paper, the mode-of-action, efficacy, and safety of a homologous RENCA cell-based vaccine in a RENCA model are described.

2. **Methods**

The experimental protocol was approved by the local Ethics Committee for Animal Experimentation, according to the United Kingdom Coordinating Committee on Cancer Research Guidelines.

2.1. **Animals**

Weight-stratified female BALB/c mice (18 g of weight), 5–6 wk of age, were obtained from Charles River Germany (Sulzfeld, Germany). The animals were acclimatized for 2 wk and maintained in separated conventional housing with 2–3 animals per cage at constant temperature and humidity. Animals were housed and fed standard mouse diet and water *ad libitum*.

2.2. **Tumour cell lines**

RENCA cells were grown in monolayer cultures. Media were routinely changed every 3 d [10]. RENCA cells were released from the tissue flasks by treatment with 0.05% trypsin/EDTA, and viability was monitored microscopically using trypan blue staining tests. For all experiments, cells were collected during the logarithmic growth phase. MethA, a nonrelated syngeneic fibrosarcoma cell line of BALB/c origin, was grown in monolayer cultures [13]. MethA was used as specificity control target cells in $^{51}$chromium-release assays [14]. YAC-1 cells were used as NK-sensitive target cells in $^{3}H$thyminde-release assays.

2.3. **Cell culture media**

RENCA cells used for tumour transplantation and vaccine preparation were grown in RPMI 1640, supplemented with 10% fetal calf serum (FCS) and 2 mM L-glutamine and were cultured in a humidified atmosphere of 95% air and 5% carbon dioxide at 37 °C. MethA was cultured in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 2 mM L-glutamine, nonessential amino acids, 100 U/ml penicillin, 100 mg/ml streptomycin, and 10% FCS, in a humidified atmosphere of 95% air and 7.5% carbon dioxide at 37 °C.

2.4. **Vaccine preparation**

The RENCA vaccine was prepared comparable to the vaccine used in humans [8]. For this purpose, RENCA cells were incubated with 1000 U Interferon-γ (IFN-γ) per 10$^7$ cells and 730 μg tocopherol acetate at 37 °C for 2 h or were left without IFN-γ-treatment. Afterwards, the RENCA cells were thoroughly washed, resuspended in 0.9% sodium chloride solution containing 0.5% glucose, adjusted to the desired cell number, and devitalized by repeated freezing and thawing in the absence of cryoprotectors. After testing for sterility and documentation of additional quality assurance procedures, the vaccine was released for experimental use and stored in a freezer. Finally, each RENCA vaccine contained a lysate of 1 × 10$^6$, 5 × 10$^5$, or 1 × 10$^5$ cells per 200 μl, respectively (groups A–C). Also, the entire production process was performed with the addition of 730 μg tocopherol acetate but without IFN-γ (groups D–F).

2.5. **Vaccination**

Eight-week-old female BALB/c mice were repeatedly vaccinated by subcutaneous administration of the RENCA vaccine at different dosages and incubation regimens (Table 1). An additional group of 12 animals received only subcutaneous solvent and served as control group (group G). The first vaccination was performed 91 d before tumour transplantation (day -91), and vaccinations were repeated every 3 wk. The tumour transplantation itself was performed on day 0. The sixth vaccination was performed 14 d (day 14) after tumour transplantation (Table 1).

2.6. **Tumour transplantation and follow-up**

The injection of 5 × 10$^5$ RENCA cells in 40-μl aliquots into the subcapsular space of the left kidney was performed through a flank incision. The animals were anesthetized with 1.5–2 volume percent isoflurane in combination with an oxygen flow of 2 l/min. One week later, the primary tumour was macroscopically visible in solvent control mice. The following parameters were assessed during the study period: clinical signs and mortality, twice daily; inspection for tissue masses and body weight, three times a week; food consumption, weekly.

2.7. **Tumour response**

On day 21 after tumour transplantation, all mice were sacrificed for evaluation of tumour response. The animals were anesthetized by intraperitoneal injection of pentobarbitone and killed by exsanguination. Blood samples were collected and centrifuged and serum specimens were stored at −80 °C. A complete necropsy was performed and all organs inspected for metastases and other abnormalities. Volumes of the tumour-bearing kidneys and contralateral kidneys were determined by caliper measurement of the largest diameter (A) and
first perpendicular (B). Tumour volume (cm³) was calculated according to the formula 0.5 × A × B². Lung metastases were counted using a dissection microscope. Kidneys and lungs were also documented by digital photography.

2.8. T-lymphocyte (splenocyte) response

During necropsy, the spleens from all animals were removed under aseptic conditions, placed into cold sterile PBS (at 2–8°C), and processed within 12 h. Tumour-specific cytotoxicity of T-lymphocytes (CTLs) and NK activity were determined using a standard ⁵¹chromium-release assay against RENCA cells, MethA cells, and YAC-1 NK target cells. Effector cells for direct ex vivo cytotoxicity measurement were prepared by mechanical disaggregation of spleens, using tweezers, followed by intense suspension, using Pasteur pipettes. Remaining connective tissue was removed by filtration through wire meshes. Splenocytes were subsequently washed three times with PBS, counted using a Neubauer chamber after trypan blue staining to quantify non-vital cells, and finally adjusted with cell culture medium to the required cell densities. Ex vivo cytotoxicity measurements against RENCA cells. The lymphocyte subsets to be selectively depleted were natural killer (NK) cells using anti-DX5 magnetobeads (Miltenyi Biotec, Bergisch Gladbach, Germany), T helper lymphocytes (anti-CD4), cytotoxic T-lymphocytes (anti-CD8), and all T-lymphocytes (anti-CD4 plus anti-CD8). All depletions were controlled by flow cytometric analysis. Tumour challenge and follow-up was as described above. RENCA tumour growth was recorded as tumour volumes over a period of up to 50 d following challenge. At the end of the observation period, all recipient animals were assessed for metastases by gross necropsy.

2.10. Statistical analysis

Results are given as means with standard deviations (SD) or standard errors (SEM), or medians with range. A Mann-Whitney U-test was used for statistical analyses of nonparametric data. Statistical analysis of parametric cytotoxicity data was done using Analysis of Variances (ANOVA). A p-value of less than 0.05 was considered to be statistically significant.

3. Results

3.1. Clinical response

No significant changes in behaviour and no adverse clinical signs were observed in the 84 vaccinated and control animals throughout the study period of 102 d. One mouse from the control group died due to tumour burden the night before necropsy. All animals demonstrated regular increase of body weight during the period before tumour transplantation (days −91 to day 0). After tumour transplantation, animals in the RENCA vaccine groups demonstrated a stable body weight independent from vaccine dose or formulation, whereas animals from the control group experienced a significant weight loss by more than 10% (Fig. 2A and B).

3.2. Tumour response

Animals from the control group had a 100% uptake rate of primary RENCA tumours. In the vaccine groups only 7/72 animals (10%) treated with the RENCA vaccine demonstrated primary tumour growth. The mean tumour volumes (left kidney) in the vaccine groups varied between 0.2 cm³ and 0.42 cm³, compared to 2.2 cm³ in controls (Fig. 3A and B). All animals from the control group had lung metastases, coinciding with a subcutaneous challenge with 1 × 10⁶ living RENCA cells. The lymphocyte subsets to be selectively depleted were natural killer (NK) cells using anti-DX5 magnetobeads (Miltenyi Biotec, Bergisch Gladbach, Germany), T helper lymphocytes (anti-CD4), cytotoxic T-lymphocytes (anti-CD8), and all T-lymphocytes (anti-CD4 plus anti-CD8). All depletions were controlled by flow cytometric analysis. Tumour challenge and follow-up was as described above. RENCA tumour growth was recorded as tumour volumes over a period of up to 50 d following challenge. At the end of the observation period, all recipient animals were assessed for metastases by gross necropsy.

Table 1 – Animal groups and treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Treatment</th>
<th>Schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>12</td>
<td>IFN-γ RENCA (1 × 10⁶)</td>
<td>Days −91, −70, −9, −28, −7 and +14</td>
</tr>
<tr>
<td>B</td>
<td>12</td>
<td>IFN-γ RENCA (5 × 10⁶)</td>
<td>Days −91, −70, −9, −28, −7 and +14</td>
</tr>
<tr>
<td>C</td>
<td>12</td>
<td>IFN-γ RENCA (1 × 10⁷)</td>
<td>Days −91, −70, −9, −28, −7 and +14</td>
</tr>
<tr>
<td>D</td>
<td>12</td>
<td>No IFN-γ RENCA (1 × 10⁶)</td>
<td>Days −91, −70, −9, −28, −7 and +14</td>
</tr>
<tr>
<td>E</td>
<td>12</td>
<td>No IFN-γ RENCA (5 × 10⁶)</td>
<td>Days −91, −70, −9, −28, −7 and +14</td>
</tr>
<tr>
<td>F</td>
<td>12</td>
<td>No IFN-γ RENCA (1 × 10⁷)</td>
<td>Days −91, −70, −9, −28, −7 and +14</td>
</tr>
<tr>
<td>G</td>
<td>12</td>
<td>Solvent††</td>
<td>Days −91, −70, −9, −28, −7 and +14</td>
</tr>
</tbody>
</table>

* Initial total number of animals was 84; one animal in group G died one day before planned necropsy due to large tumour burden.
†† Solvent consisted of 0.9% sodium chloride containing glucose 0.5%.
metastases. The mean number of lung metastases was 100 (range 3–750) in controls compared to 4 (range 0–196) in the vaccine groups. Almost all animals treated with RENCA vaccine had a complete inhibition of lung metastases (Fig. 3C and D).

3.3. T-lymphocyte (splenocyte) response

The overall ex vivo cytotoxicity of spleen cells against the experimental tumour target cells (RENCA) was 6.5% of lysis at the maximum E:T ratio of 100:1 (Fig. 4). Mean lysis rates of spleen cells from all vaccine groups at an E:T ratio of 100:1 were significantly higher. Even at an E:T ratio of 25:1 in 3/6 vaccine groups, the difference compared to the control group was statistically significant (Fig. 4). Specific lysis of syngeneic MethA fibrosarcoma control cells was hardly detected in any of the vaccine groups. The overall ex vivo cytotoxicity of spleen cells against MethA target cells was not exceeding 2.6% of lysis at the maximum E:T ratio of 100:1. The lack of MethA-reactivity in spleen cells from vaccinated mice indicates tumour-specificity of the lytic response, and thus the involvement of tumour-specific CTLs. There was no correlation between cytotoxic T cell response and vaccine dose or formulation.

A significantly higher lytic activity of spleen cells against the NK cell sensitive allogeneic lymphoma YAC-1, as compared to controls, was seen in 3/6 vaccine groups (lysates corresponding to 1 × 10^7 RENCA cells without IFN-γ incubation, 1 × 10^6 RENCA cells with IFN-γ incubation, and 1 × 10^7 RENCA cells with IFN-γ incubation). In all three groups, a p < 0.01 was reached up to an effector:target (E:T) ratio of 50:1. The enhanced NK activity in those three vaccine groups must be considered as vaccine-induced.
3.4. Adoptive immune transfer

As depicted in Fig. 5A, transfer of nonfractionated spleen cells from RENCA-vaccinated donor mice into RENCA tumour-bearing recipient mice substantially reduced tumour growth, as compared with transferred spleen cells from nonvaccinated saline control mice. In contrast, transfer of sera from RENCA-vaccinated donor mice into RENCA tumour-bearing recipient mice had no effect on tumour growth (Fig. 5B), indicating that antibodies are not involved. Depletion of CD8-positive T-lymphocytes from the donor spleen cells of RENCA-vaccinated mice completely abolished the transferable antitumoural effect (Fig. 5C). No such effect of CD8 depletion was seen with spleen cells from nonvaccinated saline control mice (Fig. 5D). Up to now, our data do not indicate a protective effect of adoptively transferred CD4+ or NK cells (data not shown). The data clearly demonstrate that the antitumoural effective immune component is cell-based and mainly attributable to CD8+ T-lymphocytes. Due to the limited tumour material available, as well as the difficulties to purge T cell preparations from residual viable tumour cells, no adoptive transfer experiments were conducted with tumour-infiltrating lymphocytes from vaccinated tumour-bearing mice.

4. Discussion

Recently, we have published results from a randomized phase-III trial comparing radical nephrectomy plus an adjuvant RCC vaccine (vaccine group) and radical nephrectomy alone (control group) in patients with RCC larger than 2.5 cm. At 70 mo, the progression-free survival was 72% in the vaccine group compared to 59.3% in the control group [8]. The vaccine used for the adjuvant treatment in this clinical trial was a lysate of autologous renal tumour cells incubated with IFN-γ before lysis (Reniale). Reniale is designed as a multiepitope vaccine, intending to expose the patient’s immune system to as many relevant tumour antigens as possible. The previous clinical phase-III trial was focused on efficacy and did not investigate end-points relating to the mode-of-action of the vaccine. For elucidation of the mode-of-action of Reniale in patients, a separate, focused clinical mode-of-action trial is planned.

In the nonclinical experiments, we investigated the mode-of-action of a homologous RENCA cell-based vaccine.
in a murine model, in order to identify potential immune parameters for a later clinical mode-of-action trial. The RENCA model is considered to be the best available preclinical model reflecting the situation in RCC patients with minimal disease after total nephrectomy. The investigation presented provides good evidence that a subcutaneously administered RENCA lysate, manufactured in a process comparable to the human Reniale vaccine, is able to induce an antitumoural immune response and is effective in reducing the number of mice developing a primary tumour, as well as number of mice developing lung metastasis. Furthermore, our adoptive transfer experiments unequivocally demonstrate that the effective immune component in the murine model is tumour-specific CD8\(^+\) T-lymphocytes. Activation of NK cells, which has also been observed in some vaccination groups by ex vivo measurement of cytotoxicity, could not be confirmed as an effector mechanism by adoptive transfer. The excellent tolerability of the murine vaccine in mice corresponds well to the finding that in the phase-III trial vaccine-related side-effects occurred in less than 2\% [8].

It must be noticed that, in the clinical situation, the vaccine was administered as an “adjuvant” therapy. In the nonclinical model investigated in this study, vaccination starts before the tumourigenesis (prophylactic setting) and continues without resection of any primary lesion. This makes it a somewhat different scenario. Concomitant tumour immunity is a well known phenomenon for RCC and has been confirmed by a variety of clinical data [16–18]. There is no hint at general immunosuppression or specific peripheral tolerance in RCC patients [19]. Thus, it must be assumed that the immune system of RCC patients in the phase III trial had already been primed by tumour antigens before first exposure to the vaccine. In the murine model, the mice were naïve to the tumour antigens, and an immune

![Fig. 4 – Ex vivo cytotoxicity of spleen cells from vaccine-treated and control mice against different target cells (day 21 after tumour transplantation). Cytotoxicity of spleen cells from BALB/c mice immunized with RENCA lysates corresponding to 10\(^6\) (circles), 5 \times 10\(^6\) (squares), 10\(^7\) (triangles) tumour cells or solvent only (open squares), was measured at different E:T ratios against three different target cells. All data points represent means ± SD from N = 12 animals per group (Unpaired \(t\)-test at E:T = 100. *** \(p < 0.001\); ** \(p < 0.01\); * \(p < 0.05\); n.s.: not significant).](image-url)
response had to be generated from scratch. Nevertheless, it may be assumed that, at latest after the first vaccination of mice, the situation was comparable to the situation in patients, which means boosting and maintenance of an already primed immune response.

Currently, we do not know to what extent the immune response in patients treated with Reniale is composed of a maintained immune response of already primed effector cells, or a new immune response involving additional tumour antigens that have previously not been involved. In this context, the murine vaccine has proven to be able to induce an immune response from scratch in naïve animals, which requires more potency than maintaining an already primed response. Thus, involvement of additional antigens after vaccination may be possible in the clinical situation.

Our experimental design does not answer the question of how long the antitumoural state lasts after the last vaccination, or if there is immunologic memory induced by the vaccination. We presently hypothesize that a tumour needs a certain dimension, and sufficient areas of necrosis to release enough antigens and “danger signals” to initiate and maintain a concomitant immune response. Once the primary tumour is surgically removed, the restimulation of the immune response comes to an end, and hidden micrometastases, which are themselves too small to restimulate an immune response, are no longer under immunologic control. The main effect of the vaccination is
to keep the concomitant immune response alive by reboosting it after resection of the primary tumour, and to prolong the phase of immune control over the micrometastases. Since tumour cells are not professional antigen-presenting cells themselves, the vaccination has to assure that activated T-effector cells are continuously delivered. T memory cells, that have possibly been induced during a concomitant immune response against the primary tumour, may be of little relevance in this context, as the murine vaccine has been shown to be able to induce an immune response from the scratch.

Treatment of human tumour cells with IFN-γ has been shown by others to increase expression of MHC class I and II [20–22]. Also, intracellular adhesion molecule-1 (ICAM-1) on tumour cells is up-regulated by IFN-γ [23–25]. Thus, IFN-γ treatment is supposed to increase the antigenicity of human renal carcinoma cells and to overcome some kind of immune escape phenomenon in RCCs [26,27]. Incubation of the tumour cells with IFN-γ in case of Reniale was intended to increase the immunogenicity of the cells, not primarily by increasing MHC expression, which is rather irrelevant for a lysate, but by stimulating the antigen-processing machinery of the tumour cells. In case of a lysate, dendritic cells (DCs) are the only antigen-presenting cells. They acquire cellular antigens and integrate danger signals from dying cells to initiate antitumour immune responses. It has been demonstrated by others that stimulation of human tumour cells with IFN-γ in case of Reniale was able to induce an immune response from the scratch.

The RENCA vaccine used in our study had antitumoural and antimetastatic efficacy in all treated animals. The study did not intend to identify a minimum effective dose, and with the range of dose levels applied in this study, the strength of the antitumoural effect was independent of the dose level of the administered RENCA vaccine and incubation with IFN-γ. The lack of effect of the IFN-γ incubation could indicate that this step does not further increase the already sufficient immunogenicity of RENCA cells used in our study. In contrast to published data [30], our own flow cytometric analyses did not indicate that short term incubation of RENCA cells with IFN-γ increases the expression of interaction molecules such as MHC class I or ICAM-1 in this cell line. Thus it can be assumed that RENCA cells are already “immunogenized” to a sufficient degree, even without IFN-γ incubation. Nevertheless, incubation with IFN-γ was included into the study as one study arm because the vaccine used in humans was also treated with IFN-γ.

In our animal model, we were able to demonstrate antitumour effects from treatment with a RENCA vaccine that consists of a lysate of syngeneic tumour cells. These findings support the results from a recently published phase-III trial showing that an adjuvant RCC vaccine (Reniale) is effective in reducing the risk of tumour progression in patients with RCC following radical nephrectomy [8]. The identified mode-of-action in the murine model is activation and maintenance of activated tumour-specific CD8+ T cells. In the clinical situation, this may happen either by restimulation of CD8+ T memory cells already induced during a concomitant immune response against the primary tumour, or induced from scratch after vaccination. The maintenance of activated CD8+ T cells by repeated vaccination, when restimulation of the concomitant immune response by the primary tumour is no longer existing, prolongs the immunologic control over micrometastases after resection of the primary tumour. The findings in the murine model may give us the opportunity to define reasonable end-points in future clinical mode-of-action trials.

**Author contributions:** Christian Doehn had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Study concept and design:** Doehn, Grossman.

**Acquisition of data:** Esser, Dreves.

**Analysis and interpretation of data:** Pauels, Stelljes.

**Drafting of the manuscript:** Doehn, Pauels, Kießig.

**Critical revision of the manuscript for important intellectual content:** Esser, Dreves.

**Statistical analysis:** Esser, Dreves.

**Obtaining funding:** Grossman.

**Administrative, technical, or material support:** none.

**Supervision:** Jocham, Kießig.

**Other (specify):** none.

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Editorial Comment on: Mode-of-Action, Efficacy, and Safety of a Homologous Multi-Epitope Vaccine in a Murine Model for Adjuvant Treatment of Renal Cell Carcinoma

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During the past few years, several studies have been performed on renal cell carcinoma (RCC) to identify the prognostic factors predictive of poor cancer-related outcome [1]. Specifically, those patients harboring RCC with lymph node metastases [2], adrenal gland invasion, vena cava thrombosis above the diaphragm, or concomitant perirenal fat and vena cava thrombosis below the diaphragm [3] are those with higher probabilities of experiencing cancer-related death. Despite significant efforts to identify the patients with the poorest prognoses, no adjuvant treatment is currently available to improve the prognosis of patients after surgery [4].

Until the development of targeted therapies, vaccine therapy has been one of the most active fields of research in RCC [5]. The concept of vaccine therapy is, theoretically, quite simple. Antigens derived from the tumor cells are recognized by antigen-presenting cells that present to cytokine working group randomized trial. J Clin Oncol 2003;21:3133–40.


CD4+ and CD8+ T lymphocytes by MHC class 1 and 2 molecules; this interaction leads to the induction and proliferation of cytotoxic T lymphocyte precursors, which will finally establish an antigen-specific population aimed at destroying cancer cells [5].

In this paper, Doehn et al showed in a murine model that the RENCA cell-based vaccine induced an antitumoral immune response, which reduced the number of mice developing a primary tumor and lung metastases and demonstrated that the effective immune component was tumor-specific CD8+ T-lymphocytes [6]. Those data support the results of a phase 3 trial published a few years ago showing a 10% advantage in 5-yr progression-free survival for those patients randomized to vaccine therapy (77.4% vs 67.8%, \( p = 0.0204 \)), with the largest survival advantage identified for pT3 RCC (reference 8 in Doehn et al [6]).

To date, vaccine therapy has to be regarded only as a hope for the future: Several randomized controlled trials are still ongoing, and the evolution of the technology will likely allow the development of new strategies to improve the efficacy of tumor vaccines, alone or in combination with the novel targeted therapies.

The number of injections, the administration route, and the optimal manufacturing technique still have discussion points. Even the very important initiation of a specific immune response is a subject for investigation; this response may be hampered by so-called T regulatory cells. Combination with other agents that attach to these cells can improve the efficacy of the vaccine.

The animal study described in this manuscript deals with an adjuvant setting for RCC and addresses several of the above-mentioned issues [5]. It can be used to optimize the approach in a patient setting. The question is whether this model, which uses established tumors, reflects the adjuvant clinical setting. This clinical trial was already performed and published some years ago. It is clear that, as of yet, no adjuvant treatment after tumor nephrectomy showed a significant reduction of recurrent disease. Adjuvant immunotherapy with interferon-\( \alpha \) and/or interleukin-2 did not have an impact on survival. Trials of adjuvant treatment with angiogenesis inhibition are ongoing. Only vaccines (tumor vaccines and heat shock proteins) showed promising results in subgroups of patients [6–8]. It is clear that a good animal model, as described by Doehn et al [5], can help us to improve the vaccine approach and increase the necessary clinical efficacy.

**References**


**Editorial Comment on: Mode-of-Action, Efficacy, and Safety of a Homologous Multi-Epitope Vaccine in a Murine Model for Adjuvant Treatment of Renal Cell Carcinoma**

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Although several new treatment options exist for renal cell carcinoma (RCC), cure is still rare in patients with recurrent or metastatic disease [1–4]. Drugs that target vascular endothelial growth factor (VEGF) or the mammalian target of rapamycin (mTOR) pathway have shown better progression-free survival data than the immunotherapeutic approaches with interferon or interleukin.

The approach of tyrosine kinase inhibition is very effective in attaching the tumor vasculature; however, due to the mode or action, stabilization of the disease is the main effect. Cure is still very rare. Prevention of recurrent disease after nephrectomy for localized tumor is a valuable approach, especially when it can be done with a good toxicity profile.

One of the major advantages of the vaccine strategy is the specific immunotherapeutic mode of action. Targeting the tumor cells with immune cells should be very effective and lacks the systemic side effects of other approaches. Unfortunately, the vaccine approaches tested so far lacked efficacy. It is still believed that optimization is possible. The number of injections, the administration route, and the optimal manufacturing technique still have discussion points. Even the very important initiation of a specific immune response is a subject for investigation; this response may be hampered by so-called T regulatory cells. Combination with other agents that attach to these cells can improve the efficacy of the vaccine.

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