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# Cancer Patients Treated with Sunitinib or Sorafenib Have Sufficient Antibody and Cellular Immune Responses to Warrant Influenza Vaccination

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## Abstract

**Purpose:** The tyrosine kinase inhibitors sorafenib and sunitinib have efficacy in several types of cancer. Recent studies indicate that these agents affect the immune system. The way it affects the immune response to influenza vaccination is unknown. The aim of this study was to elucidate the specific immune response to seasonal flu vaccination in cancer patients treated with sunitinib or sorafenib.

**Patients and Methods:** Sunitinib- or sorafenib-treated cancer patients were vaccinated against seasonal influenza with an inactivated vaccine. Healthy controls and patients with metastatic renal cell cancer (mRCC) without systemic treatment (nontreated mRCC controls) were included for comparison. Antibody responses were measured at baseline, day 8, and day 22 by a standard hemagglutination inhibition assay and cellular T-cell responses at baseline and day 8 by proliferation assay and secretion of cytokines.

**Results:** Forty subjects were enrolled: 16 patients treated with sunitinib, 6 patients with sorafenib, 7 nontreated mRCC controls, and 11 healthy controls. All patients treated with sunitinib and sorafenib developed seroprotection rates comparable with controls. Functional T-cell reactivity was observed in all groups, except for patients treated with sorafenib who showed a decreased proliferation rate and IFN-γ/IL-2 production and increased IL-10 compared with healthy controls.

**Conclusion:** We conclude that influenza vaccination should be recommended to cancer patients treated with sunitinib or sorafenib. *Clin Cancer Res; 17(13); 4541–9. ©2011 AACR.*

## Introduction

Dysfunctioning of the immune system in cancer patients has been known since long (1–4). This is shown by an increased number of regulatory T cells (Treg) in peripheral blood and tumors (5, 6), an impaired functionality of dendritic cells (DC; refs. 3, 7, 8), and changed T-cell responses (9, 10). In patients with metastatic renal cell carcinoma (mRCC), there is a shift from a T helper 1 (Th1)-mediated $CD4^+$ T-cell response, which is critical for the development of a cellular antitumor immunity, toward a Th2 response that typically mediates humoral immunity (9, 10). At least, in part, this immune dysfunction is mediated by the vascular endothelial growth factor (VEGF), which inhibits the differentiation and maturation of DC (11, 12) as well as the egress of thymic precursors from the bone marrow (13). Anti-VEGF treatment can restore DC function (11, 12) and can increase the influx of T cells into the tumor (14).

Sunitinib and sorafenib are tyrosine kinase inhibitors (TKI) of the VEGF receptor, platelet-derived growth factor receptor (PDGFR), and other receptors (15, 16). Both sunitinib and sorafenib modulate the innate and adaptive immune responses, with varying clinical consequences (6, 11, 17–23). The immunologic effects of sunitinib and sorafenib have been studied in humans as well as in murine models. It was shown that sorafenib, but not sunitinib, had a detrimental effect on DC phenotype and inhibited cytokine secretion, migration ability, and T-cell stimulatory capacity in a murine model. The proliferation rate and phenotype of T cells were not affected by sorafenib. Vaccination of mice treated with sorafenib resulted in a severe, yet reversible, inhibition of CD8 T-cell–mediated immune responses (17). Others found that sorafenib decreased the proliferation of human T cells and induced T-cell apoptosis

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**Note:** Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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## Translational Relevance

Recent studies indicate that sunitinib and sorafenib affect the immune system. The way it affects the immune response to influenza vaccination is unknown. In our study, we show that a single shot of influenza vaccination is safe and effective in mounting an antibody immune response in patients treated with sunitinib or sorafenib and this immune response is comparable with healthy controls. Standard influenza vaccination can be recommended for these patients. This is the first study that explores the antibody response and cellular immune response after influenza vaccination in patients on treatment with the tyrosine kinase inhibitor sunitinib and sorafenib.

*in vitro*. Sorafenib also inhibits T-cell–mediated immune responses (18). In contrast, mice treated with subtoxic doses of sunitinib did not show an impaired CD8 T-cell response, however, a decrease in peripheral Treg numbers was observed (17).

Sunitinib and sorafenib are used for the treatment of mRCC (24, 25). Sunitinib is also approved for second line treatment of gastrointestinal stromal tumors (GIST; ref. 26) and sorafenib for treatment of hepatocellular cancer and differentiated thyroid cancer (27, 28).

These agents increase the life expectancy of mRCC and GIST patients (24, 26). With respect to these patients, efforts should be made to maintain quality of life and to reduce comorbidities whenever possible. This includes the prevention of influenza. Influenza vaccination is indicated in individuals of elderly age or with underlying health problems, because they are at increased risk of complications or decline in physical functioning and even death (29–31). The mortality rate of influenza infection can reach 9% in patients treated in oncology (32). In cancer patients treated with chemotherapy, vaccination has shown to be safe (31, 33–36), but during chemotherapy suboptimal antibody responses and in some studies even an absence of responsiveness have been reported (31, 34, 35). However the majority of patients with malignancies respond normally after a treatment-free interval of 30 days or more (31). Currently, no data are available about the safety and efficacy of influenza vaccination in cancer patients treated with sunitinib or sorafenib.

We conducted a prospective study on influenza vaccination in patients with mRCC and GIST on sunitinib- or sorafenib-treatment who were vaccinated for influenza. The patients, in this study, fulfill the criteria for yearly influenza vaccination as recommended by the Advisory Committee on Immunization Practices (37). The primary aim of this study was to investigate the antibody response upon influenza vaccination in this group of patients. Secondary aims were to investigate cellular immune responses and tolerability of vaccination in patients treated with sorafenib and sunitinib.

## Materials and Methods

### Patients and controls

The study population consisted of the following 4 groups: mRCC or GIST patients treated with sunitinib for 4 weeks or more, mRCC patients treated with sorafenib for 4 weeks or more, mRCC patients without systemic treatment for 1 year or more (the nontreated mRCC controls), and healthy controls aged 60 years or more. Patients (age $\geq$18 years) and controls, who received an invitation for influenza vaccination by the Dutch Health Care Organization, were asked to participate in this study. Subjects were excluded from participation if they had used corticosteroids during the previous 2 weeks, had received immunotherapy or other targeted therapy in the previous year (previous imatinib use was allowed in patients with GIST), were known with an immune disorder or allergy to chicken eggs, or had symptoms of an influenza-like illness on the day of vaccination. Baseline evaluations included a medical history including previous influenza vaccinations, drug use, and a full blood count. The study was approved by the institutional review board and all participants gave their written consent.

### Study design and vaccination

This was an open-label, single center study. In the autumn of 2008, all subjects in all 4 groups were vaccinated intramuscularly with a single dose of the inactivated trivalent split influenza vaccine (Influvac Solvay Pharmaceuticals S.A. or Vaxigrip Sanofi Pasteur MSD nv), that contained hemagglutinin for each of the following 3 influenza strains: A/Brisbane/59/07 (H1N1), A/Brisbane/10/07 (H3N2), and B/Florida/4/06. Sunitinib and sorafenib were administered according to standard practice. Patients treated with sunitinib at a schedule of 4 weeks on, 2 weeks off, were vaccinated in the 3th or 4th week of the sunitinib use. Peripheral blood mononuclear cells (PBMC) and serum were collected at baseline and on days 8 after influenza vaccination. Serum samples were stored at −80°C until analysis. PBMC were isolated from heparinized venous blood by density-gradient centrifugation on lymphoprep. Cells were frozen by using a cryo 1°C freezing container (Nalgene) which was put in −80°C for 24 hours, in freezing medium consisting of 45% Roswell Park Memorial Institute (RPMI) 1640 (Cambrex Bio Science), 5% pooled human serum, 40% human serum albumin, and 10% dimethyl sulfoxide (final concentration; Sigma). Vials were stored in liquid nitrogen until use.

### Antibody response on influenza vaccination

The antibody response upon vaccination was measured by the hemagglutination inhibition (HI) test with the 3 influenza strains of the vaccine as described previously (38). Antibody titers were determined at baseline, on day 8, and day 22 after vaccination. Seroprotection was defined as antibody titer of 40 or more (31, 38). Subjects



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were considered fully protected if they had protective titers to all 3 viral strains, partially protected when having protective titers only to 1 or 2 serotypes, and nonprotected when titers were less than 40. Postvaccination seroresponses were defined as a significant (≥4-fold) increase in titers (31). The results of the tests were compared between all 4 groups as well as between patients treated with sunitinib or sorafenib and the 2 control groups.

### Cellular immune response on influenza vaccination

Analytic assays to measure the full range of T-cell responses against influenza virus have been extensively documented (39). For our study, PBMC samples collected at baseline and postvaccination (day 8) were simultaneously thawed and batch processed to test lymphocyte proliferation, lymphocyte activation, and cytokine secretion. The percentage of viable cells upon thawing was more than 85% in each patient. The ratio of viable to nonviable cells was not statistically different in the 4 experimental groups. For analysis of lymphocyte proliferation and cytokine secretion, $1.5 \times 10^5$ viable PBMC were added per well in 200 μL culture medium (RPMI 1640 supplemented with 7% pooled human serum) and incubated with the virus strains (H3N2, H1N1, and B) at a final concentration of 5 μg viral protein per milliliter. Phytohemagglutinin (PHA) stimulation at a final concentration of 1 μg/mL. was used as a positive control, and a negative control consisted of cells in a culture medium alone. Stimulation tests were carried out in triplicate. After 48 hours, supernatant was harvested to analyze cytokine production. We used the Th1/Th2 11plex Kit (eBioscience) according to the manufacturer's protocol to measure the following cytokines: IL-1β, -2, -4, -5, -6, -8, -10, and -12 (p70); TNF-α and -β; and IFN-γ. To measure cell proliferation, $^3$[H]thymidine was added after 4 days for overnight incubation. $^3$[H]thymidine incorporation was measured by using a β counter. Both cytokine production and proliferation were calculated as an index in which the outcome with stimulus (PHA or virus) was calculated relative to culture medium alone. For flow cytometric analyses, the same final concentrations of virus and PHA were applied with the exception that $1 \times 10^6$ viable PBMCs were cultured in 1 mL of culture medium and cells were harvested after 24 hours. Multicolor flow cytometric analysis was conducted with a FACS-Calibur (BD Biosciences) by using directly labeled monoclonal antibodies against the early activation marker CD69 (clone FN50), CD3 (clone SK7), CD4 (clone SK3), and CD8 (clone SK1; BD Pharmingen), all according to the manufacturer's protocol.

The results of the mean logarithmic lymphocyte proliferation, CD69 expression by $CD4^+$ cells and $CD8^+$ cells, and the cytokine secretion of PBMC of the patients groups (at baseline, on day 8, and the increase from baseline to day 8) were compared with the results of the healthy controls.

### Tolerability

The tolerability of the influenza vaccination in patients treated with sunitinib or sorafenib and in controls was evaluated by using a questionnaire at baseline and on day 22.

### Statistical methods

Given the exploratory nature of this study, group sizes were not based on power calculations. Data analysis was conducted with the use of SPSS version 16.0. The ANOVA and Kruskal–Wallis test were used to compare the 4 groups on numerical variables and simultaneously 95% CI for all pairwise differences were estimated by using Sheffé's method. The 2-tailed Fisher's exact test and the $\chi^2$ test were used to compare groups on categorical variables. The increase in titer over time for each group and type of virus was analyzed by using a linear mixed model with patients as random effect to account for the fact that each patient had been measured during several days (i.e., baseline, day 8, and day 22). Pearson correlation coefficient was used to assess correlations when a sufficiently linear association was present. For each type of virus, the *t*-test was used to compare between each patient group and the healthy controls: the baseline values, the day 8 values and the change from baseline to day 8 of proliferation of lymphocytes, and cytokine production (after log-transformation). The baseline values, the day 8 values, and the change from baseline to day 8 of cytokine production were compared over all 3 types of viruses (after log transformation) between each patient group and the healthy controls. Finally, a linear mixed model was used with patients as random effect to account for the fact that each patient had been measured for these 3 types of viruses. All statistical tests were 2 sided, and $P \leq 0.05$ was considered significant.

## Results

### Patients and controls characteristics

From October 2008 to December 2008, a total of 40 patients and controls were included in the study. Twenty-six patients were diagnosed with mRCC, and 3 with GIST. Sixteen patients were treated with sunitinib and 6 patients with sorafenib. Seven nontreated mRCC patients and 11 healthy controls were included. At baseline, there were no significant differences between the 4 groups in gender, age, tumor type, hemoglobin concentration, and history of influenza vaccination (Table 1). In the sunitinib group, the neutrophil and monocyte numbers were significantly lower than in the nontreated mRCC controls. The median duration of treatment with sunitinib or sorafenib at the time of vaccination was 10 months (range 1–24) and 4 months (range 1–24), respectively.

Thirty participants (75%) received Vaxigrip and 10 (25%) Influvac. Serious side effects of the vaccinations were not reported by any of the study participants. During a follow-up of 4 months, none of the participants



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**Table 1.** Baseline characteristics of the study groups at baseline, prior to vaccination

| Characteristic | Healthy controls ($n = 11$) | | Nontreated mRCC controls ($n = 7$) | | Sunitinib-treated patients ($n = 16$) | | Sorafenib-treated patients ($n = 6$) | | *P* |
|---|---|---|---|---|---|---|---|---|---|
| | Frequency | (%) | Frequency | (%) | Frequency | (%) | Frequency | (%) | |
| Sex | | | | | | | | | |
| Male | 5 | 45 | 5 | 71 | 13 | 81 | 5 | 83 | 0.201[a] |
| Female | 6 | 55 | 2 | 29 | 3 | 19 | 1 | 17 | 0.201[a] |
| Age, y | | | | | | | | | |
| Mean | 65 | | 59 | | 59 | | 64 | | 0.214[b] 0.233[c] |
| Range | 61–73 | | 47–68 | | 37–76 | | 51–74 | | |
| Performance status | | | | | | | | | |
| 0–1 | 11 | 100 | 7 | 100 | 100 | | 6 | 100 | 1.000[a] |
| 2 | | | | | | | | | |
| Nephrectomy | | | | | | | | | |
| Yes | | | 7 | 100 | 13 | 81 | 6 | 100 | 0.257[a,d] |
| No | | | | | 3 | 19 | | | |
| Not applicable | 11 | 100 | | | | | | | |
| Prior immunotherapy | | | | | | | | | |
| Yes | | | 2 | 29 | 4 | 25 | 3 | 50 | 0.5219[a,d] |
| No | | | 5 | 71 | 12 | 75 | 3 | 50 | |
| Mean leukocyte count ($\times 10^9$/l) | 6.3 | | 6.7 | | 4.8 | | 6.1 | | 0.0341[b] 0.042[c] |
| Mean absolute neutrophil count ($\times 10^9$/l) | 3.6 | | 4.4 | | 2.6 | | 4.1 | | 0.0143[b] 0.0196[c] |
| Mean absolute monocyte count ($\times 10^9$/l) | 0.4 | | 0.5 | | 0.3 | | 0.3 | | 0.0344[c] |
| Mean absolute lymphocyte count ($\times 10^9$/l) | 2.1 | | 1.6 | | 1.7 | | 1.3 | | 0.200[b] |
| Mean LDH (U/L) | | | 398 | | 525 | | 461 | | 0.1124[b] 0.164[c] |
| Influenza vaccination prior to 2008 (%) | | | | | | | | | |
| Yes | 6 | 55 | 5 | 71 | 10 | 63 | 2 | 33 | 0.969[a] |
| No | 4 | 36 | 2 | 29 | 5 | 31 | 1 | 17 | |
| Unknown | 1 | 9 | | 1 | 1 | 6 | 3 | 50 | |

Abbreviation: LDH, lactate dehydrogenase.
[a]$\chi^2$ test.
[b]ANOVA.
[c]Kruskall Wallis.
[d]Only tested between the patients groups.

were admitted to the hospital for influenza-related complications.

### Antibody response on influenza vaccination

Vaccination induced a significant increase in HI antibody titers in all 4 study groups (Fig. 1; $P = 0.0001$). No significant differences between the groups were observed (Table 2). As expected, prior to vaccination protective titers were already observed in all 4 study groups. Compared with the controls, the sunitinib and sorafenib group showed similar seroprotection rates against all 3 virus strains, at baseline, and after vaccination. Participants who had previously been vaccinated had a significantly higher seroprotection rate ($P = 0.031$) after vaccination. Three sunitinib-treated patients had high HI titers for the H1N1 virus after vaccination (2,560–5,120; data not shown). There was no difference in the duration of sunitinib treatment between those 3 patients and the other



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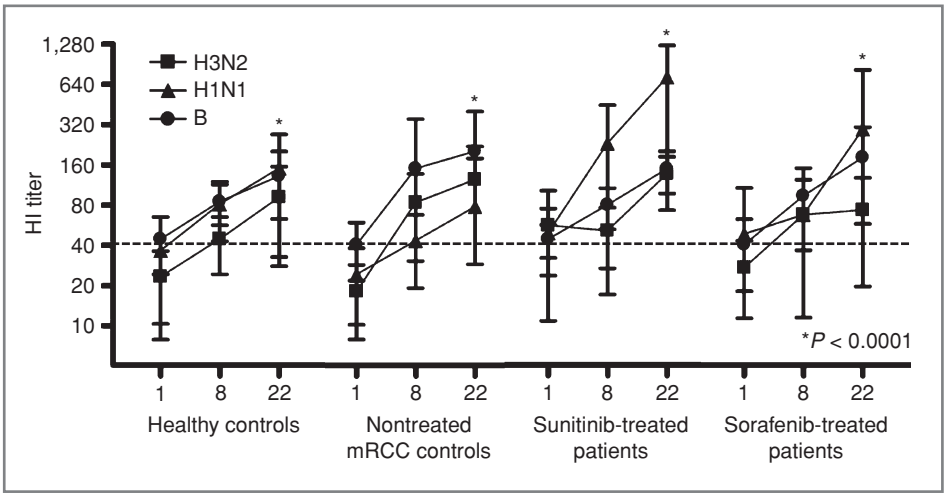


**Figure 1.** Antibody responses to influenza vaccination. Mean antibody titers by HI before (day 1) and after vaccination (day 8 and day 22). Antibody responses against all 3 vaccine strains are shown. The dotted line indicates the protective titer cutoff value. Error bars represent 95% CI. A significant increase in titers after vaccination is observed in all 4 studied groups ($P < 0.0001$).

sunitinib-treated patients. Furthermore, no correlation was observed between the duration of sunitinib or sorafenib treatment and the level of the HI antibodies, the response rate for each individual virus strain, or the increase in titer from baseline to day 22 for each virus. As expected, there was no difference in antibody response between the Influvac and the Vaxigrip vaccine.

### Cellular immune responses upon influenza vaccination

In addition to B-cell–mediated responses, as measured by HI antibody titers, vaccination also results in a T-cell response. Therefore, the proliferative capacity, cytokine secretion, and the expression of the early activation marker CD69 on T cells were investigated.

### Baseline

Before vaccination, T cells proliferated upon PHA and virus stimulation in all patient groups and healthy controls, indicating functional T cells in all study participants (Supplementary Fig. S1; PHA data not shown). This proliferation coincided with the expression of CD69 on both $CD4^+$ and $CD8^+$ cells (Supplementary Fig. S2A and B; $CD8^+$ data not shown) and the production of

**Table 2.** HI antibody response

| Seroprotection rate prevaccination | Healthy controls $n = 11$ | | Nontreated mRCC controls $n = 7$ | | Sunitinib-treated patients $n = 16$ | | Sorafenib-treated patients $n = 6$ | | $P^a$ |
|---|---|---|---|---|---|---|---|---|---|
| | Frequency | (%) | Frequency | (%) | Frequency | (%) | Frequency | (%) | |
| H3N2 | 1 | 9 | 1 | 14 | 4 | 25 | 3 | 50 | 0.255 |
| H1N1 | 4 | 36 | 3 | 42 | 8 | 50 | 3 | 50 | 0.904 |
| B | 6 | 55 | 5 | 71 | 11 | 69 | 4 | 67 | 0.859 |
| Protected (all 3 viruses) | 1 | 9 | 1 | 14 | 2 | 13 | 2 | 33 | 0.580 |
| Seroprotection rate postvaccination | | | | | | | | | |
| H3N2 | 7 | 64 | 6 | 86 | 13 | 81 | 4 | 67 | 0.628 |
| H1N1 | 9 | 82 | 5 | 71 | 14 | 88 | 4 | 67 | 0.666 |
| B | 11 | 100 | 6 | 86 | 16 | 100 | 6 | 100 | 0.184 |
| Protected (all 3 viruses) | 6 | 55 | 3 | 42 | 12 | 75 | 3 | 50 | 0.604 |
| Seroresponse rate postvaccination | | | | | | | | | |
| H3N2 | 2 | 18 | 4 | 57 | 4 | 25 | 1 | 17 | 0.265 |
| H1N1 | 4 | 36 | 1 | 14 | 4 | 25 | 1 | 17 | 0.704 |
| B | 4 | 36 | 2 | 29 | 5 | 31 | 2 | 33 | 0.987 |

Note: HI antibody response upon vaccination. Values at baseline (before vaccination) and on day 22 after vaccination. Seroprotection was defined as an antibody titer of 40 or more. Subjects were considered fully protected if they had protective titers to all 3 viral strains, partially protected when having protected titers only to 1 or 2 virus types, and nonprotected when titers were less than 40. Postvaccination seroresponses were defined as a significant ($\geq$ 4-fold) increase in titer. The results were compared between the groups with the $\chi^2$ test ([a]).



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**Table 3.** Lymphocyte proliferation after stimulation with virus strains in patients and healthy controls

| | Healthy controls $n$ =11 | | Nontreated mRCC controls $n$ = 7 | | | Sunitinib-treated patients $n$ = 16 | | | Sorafenib-treated patients $n$ = 6 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean proliferative index[a] | Range | Mean proliferative index[a] | Range | $P$ versus HC | Mean proliferative index[a] | Range | $P$ versus HC | Mean proliferative index[a] | Range | $P$ versus HC |
| H3N2 | | | | | | | | | | | |
| Baseline | 5.07 | 3.40–6.04 | 5.52 | 4.63–7.03 | 0.319 | 4.80 | 3.40–6.21 | 0.416 | 4.09 | 2.40–6.26 | 0.091 |
| Day 8 | 5.56 | 4.65–6.32 | 5.52 | 4.57–6.68 | 0.908 | 5.28 | 3.99–7.06 | 0.329 | 4.86 | 4.58–5.61 | 0.010 |
| Day 8-baseline | 0.48 | −0.89–2.92 | 0.00 | −0.88–1.30 | 0.301 | 0.48 | −0.42–3.48 | 0.992 | 0.78 | −1.57–2.39 | 0.638 |
| H1N1 | | | | | | | | | | | |
| Baseline | 5.07 | 3.47–6.49 | 5.53 | 4.57–7.17 | 0.321 | 4.88 | 3.61–6.36 | 0.581 | 4.07 | 2.48–6.03 | 0.087 |
| Day 8 | 5.42 | 4.55–6.38 | 5.34 | 3.95–6.38 | 0.831 | 5.46 | 3.91–7.98 | 0.893 | 4.87 | 4.34–5.86 | 0.064 |
| Day 8-baseline | 0.35 | −1.33–2.57 | 0.19 | −1.72–0.92 | 0.295 | 0.58 | −0.66–3.76 | 0.604 | 0.80 | −1.14–2.73 | 0.466 |
| B | | | | | | | | | | | |
| Baseline | 5.00 | 2.94–5.89 | 5.62 | 4.84–7.20 | 0.201 | 4.80 | 3.18–6.00 | 0.568 | 4.30 | 3.04–6.36 | 0.233 |
| Day 8 | 5.48 | 4.67–5.91 | 5.39 | 4.33–6.62 | 0.767 | 5.29 | 4.09–6.55 | 0.467 | 4.76 | 4.36–5.42 | 0.007 |
| Day 8-baseline | 0.48 | −1.04–2.80 | -0.23 | −1.36–0.75 | 0.152 | 0.49 | −1.03–2.24 | 0.983 | 0.47 | −1.08–2.38 | 0.978 |

Note: The mean logarithmic lymphocyte proliferation after stimulation with virus strain at base line and day 8 in the healthy controls and the patient groups (nontreated mRCC controls, sunitinib-treated patients, and sorafenib-treated patients). The difference between each of the patient groups and the healthy controls in mean proliferation from baseline to day 8 was tested with the $t$-test.
[a]The mean proliferative index is calculated as proliferation of virus-stimulated PBMC cultures relative to proliferation in PBMC cultures with only medium.

cytokines upon PHA and virus stimulation. No differences in PHA- or virus-induced T-cell proliferation and CD69 expression on T cells were observed between healthy controls and the patients groups (untreated mRCC patients and patients treated with sunitinib or sorafenib). However, patients treated with sorafenib showed a trend toward lower mean proliferation values in all measurements compared with healthy controls (Table 3; Supplementary Fig. S1; PHA data not shown). This trend coincided with a lower production of IFN-γ and IL-2 in response to PHA in this group of patients ($P$ = 0.011 and 0.024, respectively). In addition, the baseline mean IFN-γ production to all viruses (H3N2, H1N1, and B) showed a significant lower value in the sorafenib-treated group compared with healthy controls ($P$ = 0.004). Unstimulated IFN-γ production was also lower in patients treated with sorafenib ($P$ = 0.024; Fig. 2).

### Day 8

After vaccination on day 8, a lower T-cell proliferation rate was observed after stimulation with PHA ($P$ = 0.035), virus H3N2, and virus B in patients treated with sorafenib compared with healthy controls (Table 3). Furthermore, the patients treated with sorafenib showed a trend toward decreased proliferation values in all measurements compared with healthy controls (Table 3; Supplementary Fig. S1) as well as a significantly decreased expression of CD69 on $CD4^+$ cells after stimulation with virus H1N1, H3N2, and B ($P$ = 0.008, 0.026, and 0.023, respectively). Compared with healthy controls, patients treated with sunitinib also showed a lower mean CD69 expression on $CD4^+$ cells after stimulation with virus H3N2 and B on day 8 ($P$ = 0.010 and 0.012, respectively). In all 4 groups, an increased IFN-γ secretion of PBMC was observed upon influenza virus exposure *in vitro* (Fig. 2). The mean INF-γ production of PBMC in response to all viruses (H3N2, H1N1, and B) on day 8 in the sunitinib- and sorafenib-treated group showed a significant lower value compared with the healthy controls ($P$ = 0.023 and 0.002, respectively). The mean IL-10 production was significantly higher ($P$ = 0.044) in the sorafenib-treated group (Supplementary Fig. S3).

Production of IL-4, 5, and 12 and TNF-β after *in vitro* exposure of PBMC to PHA or virus strains was absent or minimal. Results for the secretion of IL-1β, 6, and 8 and TNF-α by PBMC following stimulation did not significantly differ between the 4 groups (data not shown).

After vaccination, a clear positive correlation between the humoral response and lymphocyte proliferation upon virus stimulation was observed (Fig. 3).



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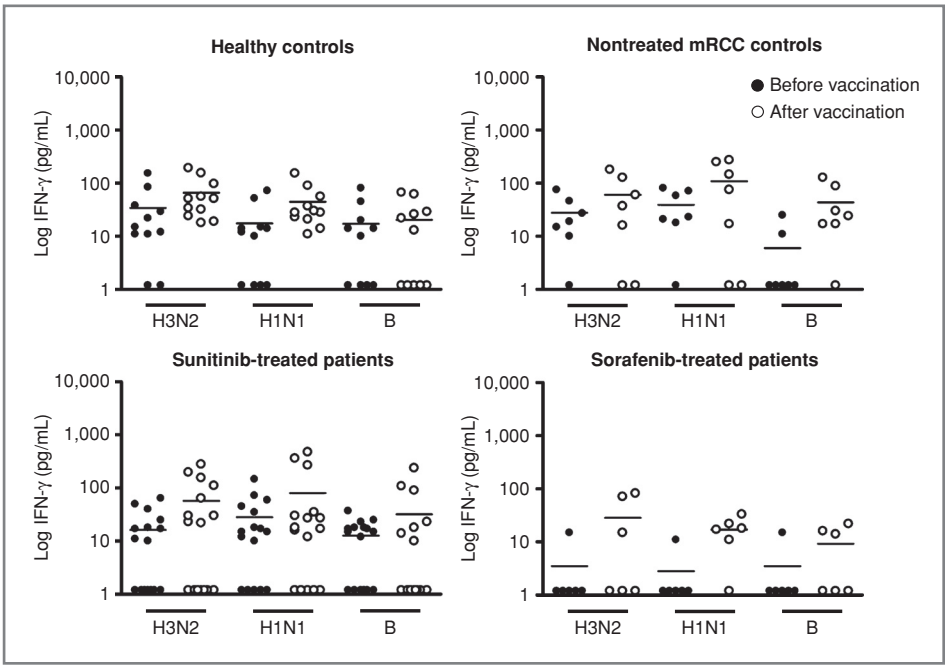


Figure 2. IFN-γ secretion in supernatants of virus-stimulated PBMC cultures shown for all 3 vaccine strains before (closed circles) and 1 week after vaccination (open circles). Mean IFN-γ secretion is indicated by horizontal lines.

## Discussion

This is the first study that explores the antibody and cellular immune responses after influenza vaccination in cancer patients treated with sunitinib or sorafenib. Our data support the use of influenza vaccination in these patients.

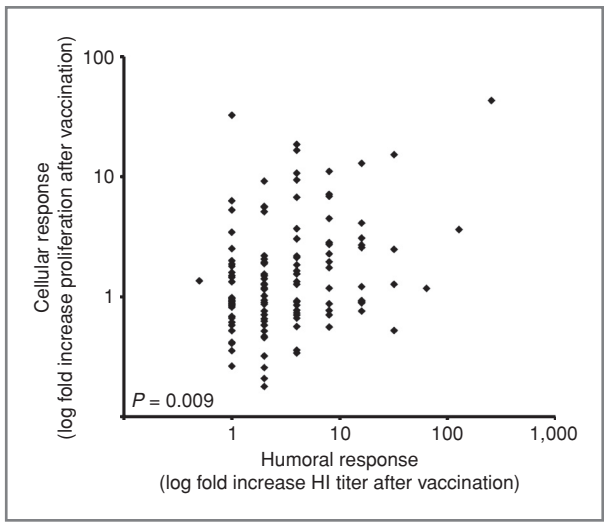


Figure 3. Correlation between humoral and cellular immune responses within patients. Vaccine-specific antibody titer (fold increase after vaccination) is correlated to the corresponding virus-stimulated PBMC proliferation (fold increase after vaccination). Each symbol represents the responses of 1 patient to a single vaccine-specific strain. A significant correlation between humoral and cellular immune responses within patients was observed ($P = 0.009$).

There is evidence that patients with cancer receiving chemotherapy are able to respond to influenza vaccination, and because this intervention is safe, inexpensive, and widely available, vaccination for seasonal influenza is indicated (31). Prevention of viral infections by influenza vaccination in cancer patients adds to the maintenance of quality of life. However, very few data are available on the clinical efficacy of influenza vaccination in cancer patients; in 1 study, a trend toward decreased morbidity and mortality upon vaccination has been observed (31). No data have been reported on the efficacy of influenza vaccination in cancer patients receiving TKI, such as sunitinib and sorafenib. This seems relevant because these agents are being used with increased frequency and are known to have immunosuppressive effects that are different from chemotherapy.

We show that cancer patients treated with sunitinib or sorafenib are able to mount an antibody response upon a single shot of influenza vaccine which is comparable with healthy controls and nontreated mRCC controls. The cellular immune responses on influenza vaccination were decreased in patients treated with sorafenib. However, in the sunitinib-treated group, we observed a cellular immune response mainly comparable with healthy controls. Controls and patients who showed a good antibody response on vaccination also showed a good cellular response and vice versa, which illustrates the consistency of our findings.

Some differences in our results between the immune responses in patients treated with sunitinib versus sorafenib warrant further discussion.

First, the cellular data showed lowest proliferative capacity in patients treated with sorafenib. This coincides with inhibition of CD69 expression and is in line with the data



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described previously (18). In contrast, we observed normal PBMC proliferation in patients treated with sunitinib. Our findings of a normal T-cell proliferative capacity in sunitinib-treated patients differ from the results of an earlier study in which a decreased proliferation was observed (19). A possible explanation is that, in that study the assays were conducted in the presence of sunitinib while we used a pooled human donor serum which was free of TKI inhibitor.

Second, PMBC from patients treated with sorafenib produced less IFN-γ and more IL-10 compared with healthy controls. In contrast, we observed that PBMC from patients treated with sunitinib before vaccination produced IFN-γ levels comparable with healthy controls suggesting a shift to a Th1 response. One of the reasons to include the nontreated mRCC patients is that mRCC patients have a shift from a Th1 to a Th2 response (9, 10). Previous studies suggest a modulating role for sorafenib and sunitinib on the Th1/Th2 balance. For sunitinib, a shift toward a Th1 immune response after treatment has been reported (6, 22, 23), whereas for sorafenib the data are more in line with a shift to a Th2 immune response (17, 18, 21). In this study, after *in vivo* stimulation of the immune system with an influenza vaccination, we obviously confirmed a more pronounced Th2 response in patients treated with sorafenib, whereas we observed some clues for a more prominent Th1 response in the sunitinib-treated patients.

A possible explanation for the difference in the immune-modulating effects of sorafenib and sunitinib is that although both these agents inhibit VEGFR and PDGFR, only sorafenib inhibits the RAF/mitogen-activated protein/extracellular signal-regulated kinase (ERK) kinase/ERK pathway (15). ERK1/2 plays a central role in natural killer (NK) cell cytotoxicity and cytokine release (40). *In vitro* pharmacologic concentrations of sorafenib, but not of sunitinib, inhibited cytotoxicity and cytokine production of resting and IL-2-activated PMBC, resulting in lower IFN-γ production from NK cells because of impaired P13K and ERK phosphorylation (21).

In summary, on the basis of our findings, a single shot of influenza vaccine is safe and effective in mounting a protective antibody response in patients treated with sunitinib or sorafenib and comparable with that in healthy controls. Therefore, standard influenza vaccination can be recommended for these patients. More studies are needed to examine the efficacy and safety of other vaccines like hepatitis A, B, or yellow fever in these patients and to explore the effects of sunitinib and sorafenib on the immune response.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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# Cancer Patients Treated with Sunitinib or Sorafenib Have Sufficient Antibody and Cellular Immune Responses to Warrant Influenza Vaccination

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