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Editor's Highlight

Real-world Data of Nivolumab for Patients With Advanced Renal Cell Carcinoma in the Netherlands: An Analysis of Toxicity, Efficacy, and Predictive Markers

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Abstract

Nivolumab has been approved as second-line treatment for advanced renal cell carcinoma in Europe. We performed a real-world analysis to validate this practice. The study included 264 patients from 24 hospitals in the Netherlands. We found that toxicity and efficacy of nivolumab are comparable with previous results. Increase in eosinophil count was the strongest predictor of improved survival. Results can be used to improve personalized therapy.

Background: Nivolumab, a programmed death 1 inhibitor, has been approved as second-line treatment for advanced renal cell carcinoma (RCC) in Europe since 2016. We investigated the toxicity and efficacy of nivolumab as well as potential predictive biomarkers in the Dutch population. **Patients and Methods:** This was a retrospective, multicenter study of the Dutch national registry of nivolumab for the treatment of advanced RCC. The main outcome parameters included toxicity, objective response rate (ORR), overall survival (OS), progression-free survival (PFS), time to progression (TTP), and time to treatment failure

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(TTF). In addition, potential predictive and prognostic biomarkers for outcomes were evaluated. **Results:** Data on 264 patients were available, of whom 42% were International Metastatic RCC Database Consortium (IMDC) poor risk at start of nivolumab, 16% had ≥ 3 lines of previous therapy, 7% had non–clear-cell RCC, 11% had brain metastases, and 20% were previously treated with everolimus. Grade 3/4 immune-related adverse events occurred in 15% of patients. The median OS was 18.7 months (95% confidence interval, 13.7-23.7 months). Progression occurred in 170 (64.4%) of 264 patients, with a 6-and 12-months TTP of 49.8% and 31.1%, respectively. The ORR was 18.6% (49 of 264; 95% confidence interval, 14%-23%). Elevated baseline lymphocytes were associated with improved PFS ($P = .038$) and elevated baseline lactate dehydrogenase with poor OS, PFS, and TTF ($P = .000$). On-treatment increase in eosinophils by week 8 predicted improved OS ($P = .003$), PFS ($P = .000$), and TTF ($P = .014$), whereas a decrease of neutrophils was associated with significantly better TTF ($P = .023$). **Conclusions:** The toxicity and efficacy of nivolumab for metastatic RCC after previous lines of therapy are comparable with the results in the pivotal phase III trial and other real-world data. On-treatment increase in eosinophil count is a potential biomarker for efficacy and warrants further investigation.

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Introduction

Nivolumab, a programmed death 1 immune checkpoint inhibitor,[1,2] interferes with the inhibition of the anti-tumor immune response, resulting in improved antitumor activity.[3] Based on the results of a randomized phase III trial comparing nivolumab with everolimus, nivolumab has been approved for the treatment of advanced renal cell carcinoma (RCC) after failure of one or several lines of vascular endothelial growth factor-targeted therapy.[4,5]

The evaluation of nivolumab in the real-world setting offers an opportunity for external validation of this pivotal phase III study, which recruited only patients with a clear-cell component and stringent selection criteria. In addition, there is a need for prognostic and predictive biomarkers in informing patients about this treatment option.

Based on studies with other cancer types treated with immunotherapy, an on-treatment increase of lymphocytes and eosinophils and a decrease of lactate dehydrogenase (LDH) and neutrophils might predict improved survival.[6-10]

This retrospective study provides an overview of toxicity, efficacy and potential predictive biomarkers in a real-world Dutch patient population with advanced RCC treated with nivolumab.

Patients and Methods

In this retrospective analysis, patients with advanced RCC treated with nivolumab at 3 mg/kg bodyweight every 2 weeks were consecutively included in a national registry commissioned by the Dutch Oncological Society (NVMO) and carried out by the Dutch Working Group on Immunotherapy of Oncology (WIN-O) after the approval of nivolumab. Data and privacy protection were based on relevant Dutch laws and regulations. Only patients with a follow-up of at least 6 months after at least 1 dose of nivolumab were included in this retrospective analysis.

The main outcome parameters of this study were toxicity, overall response rate (ORR), overall survival (OS), progression-free survival (PFS), time to progression (TTP), and time to treatment failure (TTF). Toxicity was investigator-assessed by recording grade 3/4 nivolumab immune-related adverse events (irAEs) according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 4.[11] ORR was derived from radiologic reports and assessment of investigators and were, in general, based on the immune Response Evaluation Criteria in Solid Tumors (iRECIST)-defined response criteria.[12] OS was defined as the time between starting nivolumab and death. PFS was defined as the time between starting nivolumab and documented tumor progression leading to discontinuation of nivolumab by iRECIST. TTP was defined as PFS except for the exclusion of death by other causes than progressive disease (PD). TTF was defined as time between the start and stop of nivolumab. The median duration of responses until progression or censoring was also measured (lost to follow-up after stopping treatment owing to AEs while ongoing stable disease [SD] or next lines of treatment).

Pseudo-progression was defined as unconfirmed PD followed by SD or regression. As potential prognostic and predictive markers, the following parameters were assessed at baseline: gender, age, number and sites of metastasis[13]; histologic subtype; World Health Organization (WHO) performance status[14]; Memorial Sloan-Kettering Cancer Center (MSKCC) risk score[15]; International Metastatic Renal-Cell Carcinoma Database Consortium (IMDC) risk score[16]; number of different previous systemic treatments; nephrectomy; hemoglobin (Hb), calcium, albumin, and c-reactive protein (CRP); and erythrocyte sedimentation rate (ESR). As potential on-treatment markers, lactate dehydrogenase (LDH), white blood cells (WBCs), lymphocytes, eosinophils, neutrophils, and thrombocytes were assessed at baseline and at week 4, 8, and 12. Grade 3/4 AEs occurring between baseline and week 8 were also studied as a marker.

On-treatment eosinophil and lymphocyte increase and neutrophil decrease were measured to explore a relation with efficacy of

nivolumab treatment as previously performed in other types of cancer.[6]

Statistical Analysis

Data was analyzed using SPSS statistical software version 22.0. OS, PFS, and TTF were assessed using the Kaplan-Meier method. For ORR, univariate analyses were performed using 1-sample *t* tests. Univariate analyses for OS, PFS, and TTF were also analyzed using the Kaplan-Meier method and compared by the log-rank test. Hazard ratios (HRs) were obtained by performing Cox regression analyses. Paired univariate analyses for ORR were performed with a nonparametric Wilcoxon signed rank test and Shapiro-Wilk test. The Pearson χ^2 test was used to assess the significance of the relation between potential predictive biomarkers and ORR. Data was considered of significance when the probability (*P*) was less than .05. Patients lost to follow-up were censored.

Results

Patients

Between March 2016 and January 2018, 264 patients with RCC from 24 Dutch hospitals were included in the registry and available for the analysis. Forty-two percent of patients had poor risk according to IMDC criteria, 16% of patients had 3 or more lines of previous therapy, 7% had non–clear-cell subtypes, 11% had brain metastases, and 20% were previously treated with everolimus. An overview of baseline demographics and clinical characteristics of the patients is given in Table 1. For a detailed analysis of subgroups, see Supplemental Appendix A and Supplemental Figure 1 (in the online version). At data cutoff (according to date of data collection), after a median follow-up of 12.2 months, 65 (25%) of 264 patients continued to receive nivolumab. The primary reason for discontinuation of nivolumab was disease progression (149 of 264 patients; 56%).

Toxicity

Grade 3/4 treatment-related or possibly irAEs occurred in 39 (15%) of the 264 patients. A total of 46 (17%) grade 3/4 irAEs occurred, of which 8 (3%) were possibly nivolumab-related and 38 (14%) were nivolumab-related (Table 2). Two nivolumab-related deaths were reported (1 grade 5 thrombocytopenia and 1 grade 5 pneumonitis). irAEs leading to treatment discontinuation occurred in 29 (11%) of the 264 patients, of which 6 (2%) patients temporarily discontinued nivolumab because of an ongoing response (see Table 2).

Efficacy

The median OS was 18.7 months (95% confidence interval [CI], 13.7-23.7 months). Six-, 12- and 18-month OS was 78.6%, 61.6%, and 50.8%, respectively. The median PFS was 5.6 months (95% CI, 4.2-7.1 months) with 6- and 12-month PFS of 48.8% and 29.0%, respectively. Progression occurred in 170 (64.4%) of 264 patients, with a 6- and 12-month TTP of 49.8% and 31.1%, respectively. The median TTP was 6.0 months (95% CI, 4.5-7.4 months). The ORR was 18.6% (49 of 264; 95% CI, 14%-23%). After a median follow-up of 12.3 months, 14.4% of patients had disease control (SD or

Table 1 Baseline Demographic and Clinical Characteristics of the Patients

Characteristic	Patients (N = 264), n (%)
Median age, y (range)	65 (35-88)
Gender	
Male	200 (76)
Female	64 (24)
MSKCC risk group	
Favorable	22 (8)
Intermediate	145 (55)
Poor	97 (37)
IMDC risk group[a]	
Favorable	21 (9)
Intermediate	122 (49)
Poor	104 (42)
WHO performance status	
0	85 (32)
1	142 (54)
2	33 (13)
3	4 (2)
Number of metastatic sites	
1	21 (8)
2-3	144 (55)
4-5	83 (32)
≥6	16 (6)
Sites of metastasis	
Lung	209 (79)
Lymph node	181 (69)
Soft tissue	110 (42)
Bone	108 (41)
Liver	78 (30)
Adrenal	63 (24)
Brain	28 (11)
Pancreas	28 (11)
Renal	17 (6)
Thyroid	6 (2)
Spleen	4 (2)
Stomach	4 (1)
Intestine	3 (1)
Gallbladder	2 (1)
Bladder	1 (<1)
Heart	1 (<1)
Previous nephrectomy	
Yes	185 (70)
No	79 (30)
Previous systemic treatments	
0	1 (<1)
1	161 (61)
2	61 (23)
3	28 (11)
4	9 (3)

Table 1 Continued

Characteristic	Patients (N = 264), n (%)
5	2 (1)
6	2 (1)
Previous systemic therapy	
Sunitinib	169 (64)
Pazopanib	135 (51)
Everolimus	53 (20)
Axitinib	25 (10)
Sorafenib	18 (7)
Bevacizumab/interferon	8 (3)
Interferon	6 (2)
Everolimus/cyclophosphamide	6 (2)
Sorafenib/capecitabine	3 (1)
Bevacizumab/everolimus	1 (<1)
Capmatinib	1 (<1)
Fuhrman grading	
I	8 (5)
II	57 (32)
III	72 (40)
IV	42 (24)
Histology	
Clear-cell	212 (93)
NOS	6 (3)
Papillary	5 (2)
Papillary/clear-cell	4 (2)
Papillary/collecting duct	1 (<1)
Chromophobe	1 (<1)
Primary tumor classification at diagnosis	
T1	28 (15)
T2	50 (28)
T3	94 (49)
T4	18 (9)

Abbreviations: IMDC = International Metastatic Renal Cell Carcinoma Database Consortium; MSKCC = Memorial Sloan-Kettering Cancer Center; WHO = World Health Organization.
[a]Missing data.

regression). In 14 (29%) of 49 patients with tumor response, nivolumab had been discontinued because of irAEs. Eleven (79%) of these patients had an ongoing response after discontinuation of nivolumab. The median duration of responses until progression or censoring since nivolumab was stopped was 3.8 months (range, 1.6-12.1 months). Of 49 responding patients, 20 (41%) patients had a response of at least 1 year. These durable responders received a median of 26 infusions of nivolumab (range, 8-34). See Figure 1 for an overview of the responding patients stratified for MSKCC risk group and number of previous systemic treatments. There were no differences in MSKCC and IMDC risk groups in terms of responders. However, in the MSKCC poor-risk group, responses seem less durable when compared with the favorable- and intermediate-risk group. Pseudo-progression occurred in 20% of the responding patients.

Table 2 Treatment-related Adverse Events (n = 264)

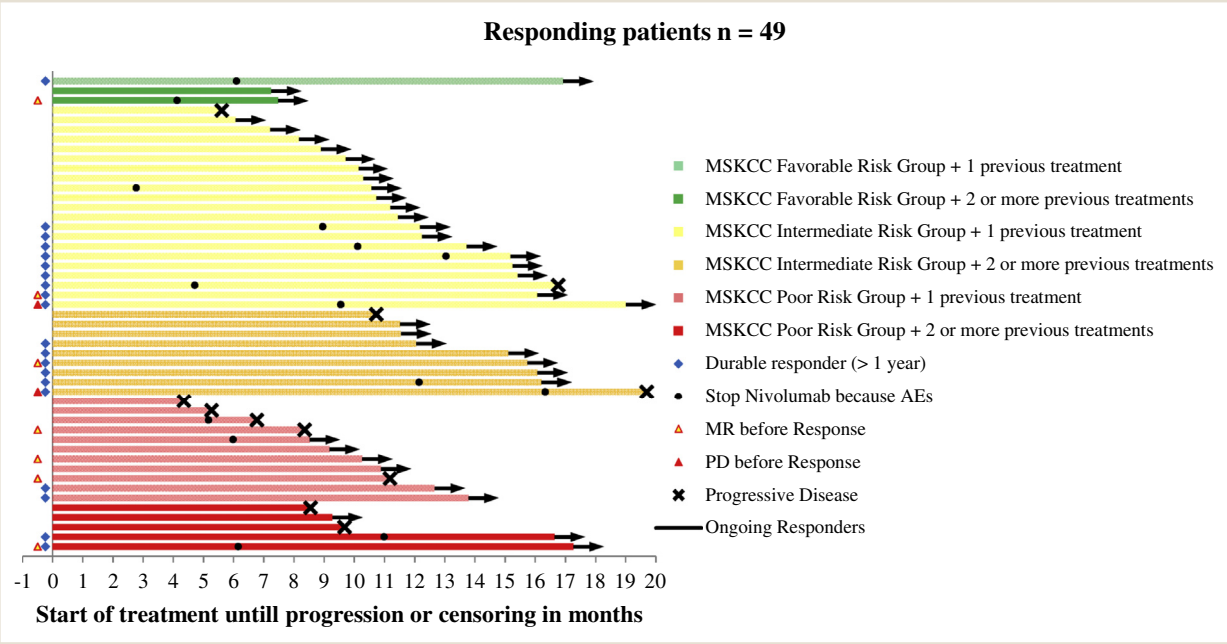
Event	Nivolumab-related Grade 3/4, n (%)	Possibly Nivolumab-related Grade 3/4, n (%)	All Grade 3/4, n (%)
All events	38 (14)	8 (3)	46 (17)
Hepatitis	9 (3)	0	9 (3)
Pneumonitis	4 (2)	2 (<1)	6 (2)
Nephritis	5 (2)	1 (<1)	6 (2)
Colitis	4 (2)	0	4 (2)
Fatigue	3 (1)	0	3 (1)
Arthritis	2 (<1)	0	2 (<1)
Myocarditis	1 (<1)	1 (<1)	2 (<1)
Dermatitis	1 (<1)	0	1 (<1)
Neuropathy	1 (<1)	0	1 (<1)
Thrombocytopenia	1 (<1)	0	1 (<1)
Peritonitis	1 (<1)	0	1 (<1)
Heart failure	1 (<1)	0	1 (<1)
Cholecystitis	1 (<1)	0	1 (<1)
Pancreatitis	1 (<1)	0	1 (<1)
Infusion reaction	1 (<1)	0	1 (<1)
DM type 1/ ketoacidosis	1 (<1)	0	1 (<1)
Encephalitis	1 (<1)	0	1 (<1)
Malaise	0	1 (<1)	1 (<1)
Nephrotic syndrome	0	1 (<1)	1 (<1)
Hypophysitis	0	1 (<1)	1 (<1)
Serositis	0	1 (<1)	1 (<1)

Abbreviation: DM = diabetes mellitus.

Assessment of Potential Predictive Markers

We examined the on-treatment changes of baseline levels of LDH, eosinophils, lymphocytes, and neutrophils between week 0 and 12, as well as WHO performance status, occurrence of AEs, and number of metastatic sites (see Supplemental Appendix A in the online version). Most of these subgroups were significant in relation to ORR ($P < .0001$, $P < .0001$, $P = .011$, $P = .322$, $P = .016$, $P = .034$, and $P = .090$, respectively). Regarding response to treatment, we found a significant increase in eosinophils between week 0 and weeks 4, 8, and 12 in both responders and non-responders, but only the changes at week 8 were significant in predicting OS, PFS, and TTF between responders and non-responders (Figure 2).The increase in lymphocyte count was significant for responders, whereas there was no significant change among non-responders (Supplemental Figure 2 and Supplemental Appendix B in the online version). Conversely, neutrophil count did not change significantly among responders, yet non-responders experienced a significant increase in neutrophils over the weeks 0 to 12 (see Supplemental Figure 3 in the online version). Similarly, the difference between LDH at week 0 and weeks 4, 8, and 12 was significant in non-responders, but not in responders (see Supplemental Appendix B in the online version). It is noteworthy that there was no significant difference in the baseline level of any of the markers between responders and non-responders.

Figure 1 Overview of the Responding Patients Stratified for MSKCC Risk Group and Number of Previous Systemic Treatments



Abbreviations: AEs = adverse events; MR = mixed response; MSKCC = Memorial Sloan Kettering Cancer Center; PD = progressive disease.

Assessment of Potential Prognostic Markers

When looking at OS, PFS, and TTF, we first examined the association between baseline levels of the markers with prognosis. We found that, although higher levels of baseline lymphocyte count were associated with better PFS when compared with lower baseline levels ($P = .038$), an elevated baseline level of LDH was associated with worse OS, PFS, and TTF when compared with normal baseline level ($P = .000$). Moreover, a significant association was found for the lowered and normal baseline neutrophil count with better OS when compared with elevated baseline neutrophil count ($P = .040$). However, there was no association between normal and elevated baseline eosinophil count in regards to OS, PFS, and TTF (see Supplemental Appendix B in the online version).

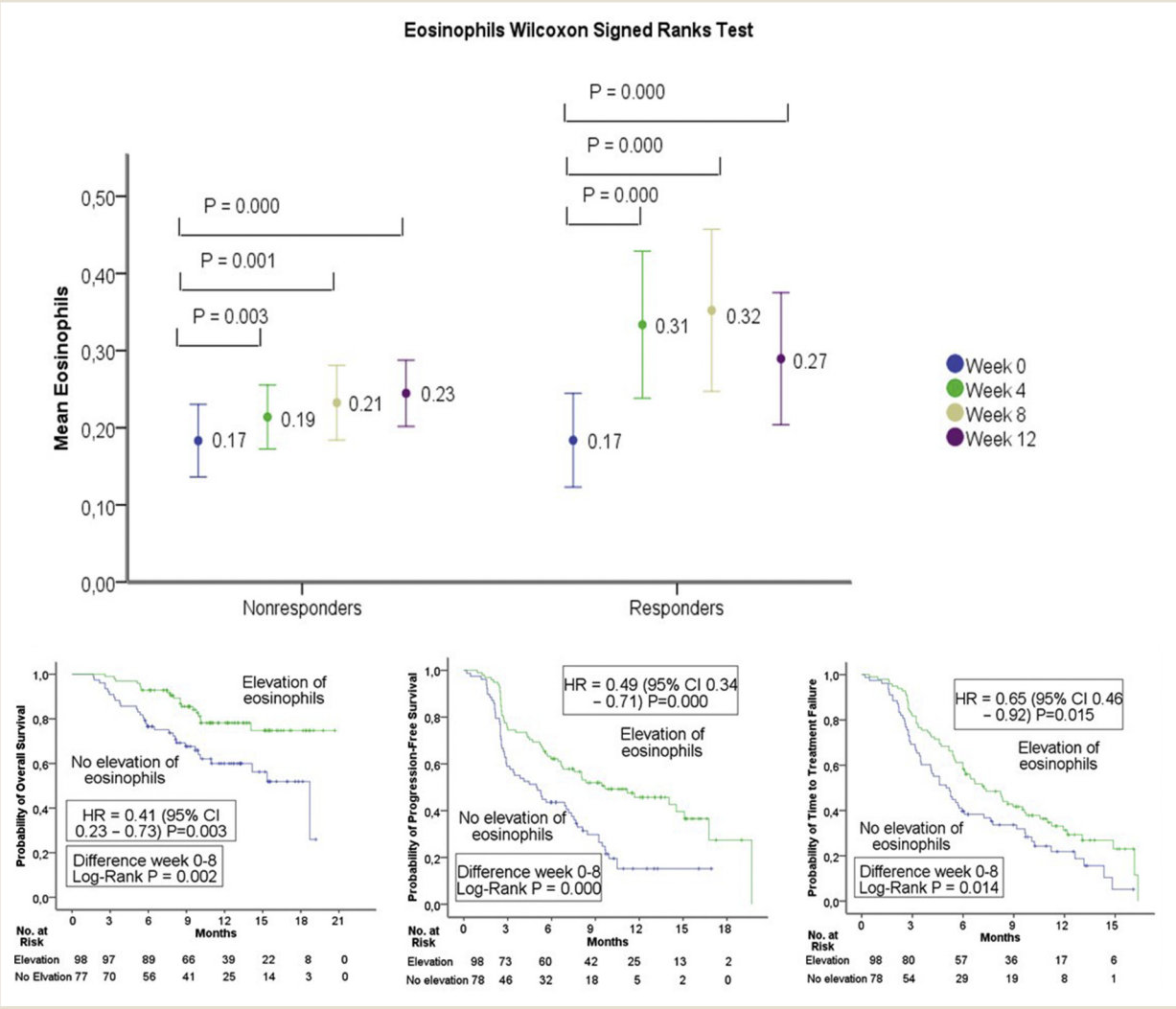
Discussion

In the current study, we examined 264 patients from 24 hospitals in the Netherlands treated with nivolumab for advanced RCC. Compared with the pivotal trial population of Motzer et al,[4] the irAE rate was similar, and the observed ORR was comparable (18.6%; 95% CI, 14%-23% and 25%; 95% CI, 21%-29%, respectively), suggesting that nivolumab is beneficial in a similar percentage of unselected patients as in the pivotal trial. However, in this cohort, the median OS was noticeably lower (18.7 months; 95% CI, 13.7-23.7 months and 25.0 months; 95% CI, 21.8 months to not estimable, respectively). Such differences were probably owing to the poorer prognosis of the real-world patient population when compared with the select trial population.

In this real-world data analysis, patients had an overall poorer performance score, higher tumor load, and poorer MSKCC risk score, whereas in the CheckMate 025 trial, 35% of patients had a favorable risk, 49% an intermediate risk, and only 16% a poor risk by MSKCC. Interestingly, the median OS in the real-world data was comparable with the median OS of the everolimus group in the CheckMate 025 trial (19.6 months; 95% CI, 17.6-23.1 months), but higher than previously reported data for second-line treatment with everolimus.[17] Notably, the reported PFS in this cohort was slightly longer than in the pivotal trial (5.6 months; 95% CI, 4.2-7.1 months and 4.6 months; 95% CI, 3.7-5.4 months, respectively), which could be explained by a lack of protocolized imaging in the retrospective study. TTF and TTP were added as primary objectives to underpin the results of PFS.

In 2018, Albiges et al[18] presented a prospective study regarding the safety and efficacy of nivolumab in the real-world French patient population with advanced RCC (n = 528). Equivalent to the current analysis, patients were included after previous treatment with a mammalian target of rapamycin inhibitor, a WHO performance score of 2, and asymptomatic brain metastases. Thus, patients with non–clear-cell histology were still excluded. Patients had an overall better risk score stratification, as 19%, 55%, and 26% of patients were in the favorable, intermediate, and poor IMDC risk groups, respectively. Fourteen percent of their patients had symptomatic brain metastases at baseline compared with 11% in the current cohort. Interestingly, with similar irAE rates, our median PFS, 1-year OS, and median OS were all comparable with the French real-world patient population,[18] which was 4.4 months (95% CI, 3.0-4.6 months), 66.4%, and 18.6 months (95% CI, 16.0-18.6 months), respectively. In the French study, 31% of patients still received nivolumab at a median follow-up of 13.1

Figure 2 Difference in Baseline Eosinophil Level Between Responders and Nonresponders



Abbreviations: CI = confidence interval; HR = hazard ratio.

months, compared with 25% at a median follow-up of 12.2 months in the current study. It is remarkable that, after a median follow-up of 12.3 months, 78% of responders are still having an ongoing response, giving hope for durable responses. In support of these findings, a study by McDermott et al found a median duration of response of 22 months after a follow-up of 38 months in a phase II study with 167 patients, with a 3-year OS rate of 35%.[19]

To date, there is no clear guidance regarding the duration of treatment or whether nivolumab should be ceased after a certain number of infusions. In the current study, 14 (29%) of the 49 responding patients discontinued nivolumab owing to irAEs. Eleven (79%) of those are ongoing responders after nivolumab was stopped, with a median treatment time of 8 months (range, 3-16 months). Such findings suggest that shorter duration of treatment may be sufficient for some patients. Early detection of such patients could potentially lead to fewer irAEs and lower costs, supporting further research in this field. Conversely, early treatment discontinuation owing to progression should be done with caution. In the current analysis, pseudo-progression occurred in up to 20% of the responding patients. Therefore, it is recommended to continue nivolumab until PD is confirmed by subsequent imaging. To be able to make predictions whether patients with unconfirmed PD might still develop a tumor response, predictive biomarkers are needed. However, given the retrospective nature of the study, we do not know if pseudo-progression was confirmed in each individual patient.

Further analysis of the current data revealed a response to treatment across all stratified subgroups, divided by MSKCC risk group and number of previous systemic treatments. However, responses seem less durable in the MSKCC poor-risk group, whereas OS, PFS, TTP, and TTF were significantly longer in the favorable-risk group followed by the intermediate-

risk group. When stratifying by number of previous systemic treatments, it seems that the number of treatments did only affect the efficacy of nivolumab in terms of OS, which was better in patients who received > 1 line of therapies. One potential explanation could be that patients who were able to receive more lines of treatment probably had better performance status and hence longer OS. However, in the French real-world data, more than 2 previous systemic treatments, as well as prior treatment with everolimus, were associated with poorer OS.[18] Contrary to the pivotal study and the French data, our analysis included patients with non−clear-cell RCC. Although this made up only 7% of the cohort, we found that non−clear-cell histology is associated with poorer median OS, PFS, TTP, and TTF, compared with clear-cell RCC.

In patients with other types of cancer treated with immunotherapy, both on-treatment increases of lymphocytes and eosinophils were found to be predictive markers for efficacy. Our data corroborate this finding by showing significant associations with ORR for both increases in lymphocytes and eosinophils. On-treatment increase in eosinophils had also a strong significant association with OS, PFS, and TTF. Furthermore, a decrease in neutrophil count and a better WHO performance status were significant markers for efficacy. Also, elevated baseline LDH, which is widely known as a prognostic factor, was strongly associated with poorer OS, PFS, and TTF. However, for the changes in WBC counts between weeks 0 and 8, validated standards have not been reported. Therefore, these potential biomarkers need to be validated in further studies with a control group. Despite the limitations, baseline and on-treatment routine WBCs may have potential as a marker for efficacy and response in patients receiving nivolumab.

This study has several limitations owing to its retrospective nature. The irAE and objective responses, as well as PFS, were not assessed under protocolized trial conditions and without an independent central review. Also, only the start and final end date of nivolumab were taken into account in this study. Personalized information on temporary delay or discontinuation of treatment was not registered and unavailable. Therefore, TTF and TTP were included as primary objectives to confirm results found in relation to PFS. Although we provided a flowchart with guidelines for assessment of clinical and laboratory parameters during nivolumab treatment, it was not followed in all instances.

In conclusion, this study gives an overview regarding the toxicity, efficacy, and potential predictive biomarkers of nivolumab in the Dutch advanced RCC population. These results consolidate the results of the pivotal phase III CheckMate 025 study and real-world French data. Personalized therapy requires validated biomarkers for each treatment option.

On-treatment increase in eosinophil count by week 8 was the strongest predictor of improved OS, PFS, and TTF, and may serve as predictive marker for nivolumab after previous lines of therapy.

- Nivolumab, a programmed death 1 immune checkpoint inhibitor, interferes with the inhibition of the anti-tumor immune response, resulting in improved antitumor activity. Based on the results of a randomized phase III trial comparing nivolumab with everolimus, nivolumab has been approved as second-line treatment for advanced RCC in Europe since 2016. In the current study, we investigated the toxicity and efficacy of nivolumab as well as potential predictive biomarkers in the Dutch population with advanced RCC treated with nivolumab.
- The study included 264 patients from 24 hospitals in the Netherlands treated with nivolumab for advanced RCC. Results supported previous studies including the pivotal phase III study and other real-world data. With regard to predictive biomarkers, we found that on-treatment increase in eosinophil count was the strongest predictor of improved survival and may serve as a predictive marker for nivolumab after previous lines of therapy.
- This current evaluation of nivolumab in the real-world setting offers an opportunity for external validation of this practice and offers a biomarker for progression that can be used to improve personalized therapy.

Disclosure

The authors have stated that they have no conflicts of interest.

CRediT authorship contribution statement

Saskia Lisa Verhaart: Writing - original draft, Formal analysis. **Yasmin Abu-Ghanem:** Writing - original draft. **Sasja F. Mulder:** Writing - review & editing. **Astrid Van Der Veldt:** Writing - review & editing. **Susanne Osanto:** Writing - review & editing, Writing - review & editing. **Maureen J.B. Aarts:** Writing - review & editing. **Danny Houtsma:** Writing - review & editing. **Frank P.J. Peters:** Writing - review & editing. **Gerard Groenewegen:** Writing - review & editing. **Carla M.L. Van Herpen:** Writing - review & editing. **Loes M. Pronk:** Writing - review & editing. **Metin Tascilar:** Writing - review & editing. **Paul Hamberg:** Writing - review & editing. **Maartje Los:** Writing - review & editing. **Gerard Vreugdenhil:** Writing - review & editing. **Marco Polee:** Writing - review & editing. **Albert J. Ten Tije:** Writing - review & editing. **John B.A.G. Haanen:** Conceptualization, Writing - review & editing. **Axel Bex:** Writing - review & editing. **Alfonsus J. van den Eertwegh:** Methodology, Conceptualization.

Supplemental Data

Supplemental figures accompanying this article can be found in the online version at https://doi.org/10.1016/j.clgc.2020.10.003.

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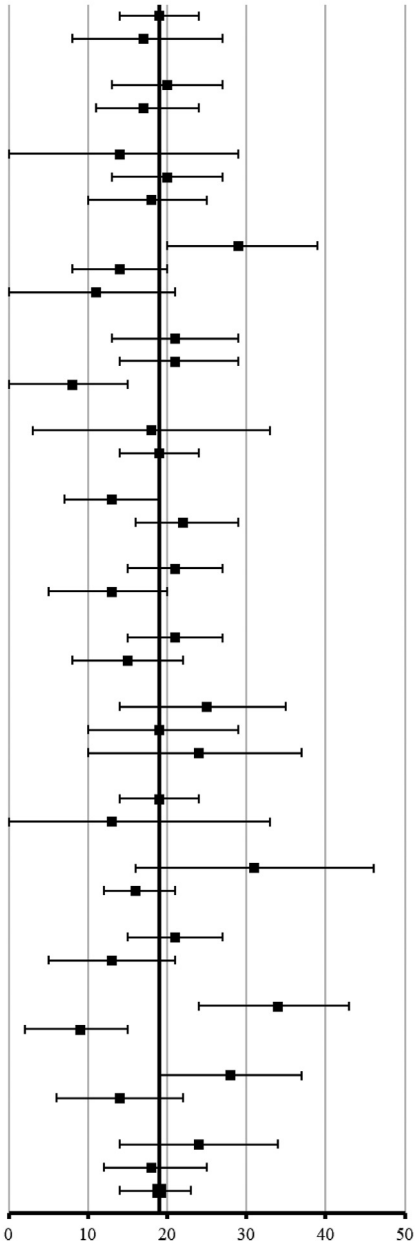
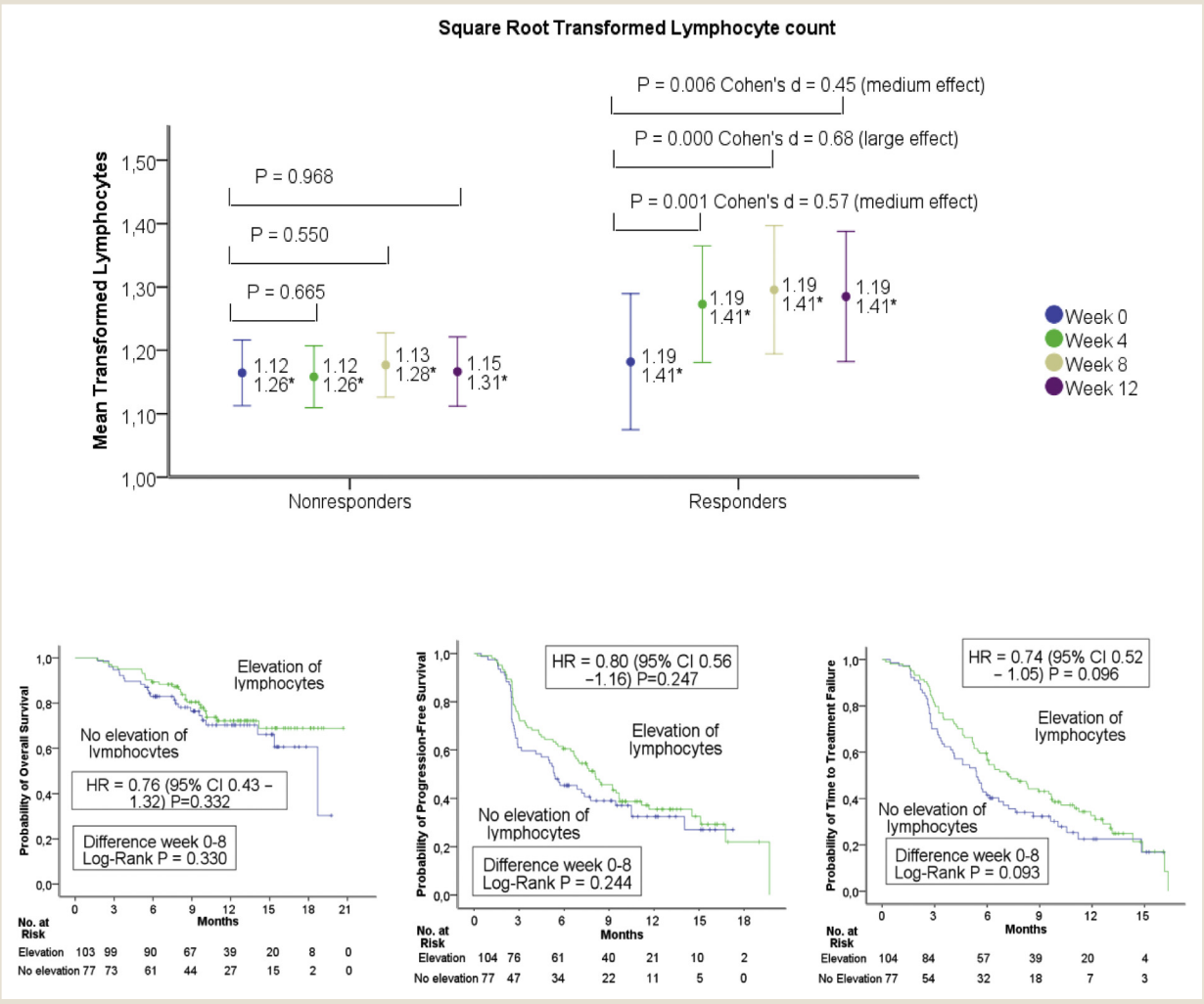
Supplemental Figure 1 Objective Response Rate in Subgroups

Source	No. With Objective Response	Total No. Of Patients	Objective Response Rate, % (95% CI)
Sex			
Male	38	200	19 (14-24)
Female	11	64	17 (8-27)
Age			
< 65	25	126	20 (13-27)
≥ 65	24	138	17 (11-24)
MSKCC risk group			
Favorable	3	22	14 (0-29)
Intermediate	29	145	20 (13-27)
Poor	17	97	18 (10-25)
WHO performance status			
0	25	85	29 (20-39)
1	20	142	14 (8-20)
≥ 2	4	37	11 (0-21)
Number of sites of metastasis			
1-2	20	96	21 (13-29)
3-4	25	117	21 (14-29)
≥ 5	4	51	8 (0-15)
Brain metastases			
Yes	5	28	18 (3-33)
No	44	236	19 (14-24)
Bone metastases			
Yes	14	108	13 (7-19)
No	35	156	22 (16-29)
Previous Nephrectomy			
Yes	39	185	21 (15-27)
No	10	79	13 (5-20)
Previous systemic treatments			
≤ 1	34	162	21 (15-27)
≥ 2	15	102	15 (8-22)
Fuhrman grading			
I-II	16	65	25 (14-35)
III	14	72	19 (10-29)
IV	10	42	24 (10-37)
Histology			
Clear-cell	45	236	19 (14-24)
Other	2	15	13 (0-33)
Severe adverse events			
Yes	12	39	31 (16-46)
No	37	225	16 (12-21)
Baseline LDH			
Normal < ULN	40	189	21 (15-27)
Elevated ≥ ULN	9	71	13 (5-21)
Eosinophil change week 0-8			
Elevated	33	98	34 (24-43)
Not elevated	7	78	9 (2-15)
Lymphocyte change week 0-8			
Elevated	29	104	28 (19-37)
Not elevated	11	77	14 (6-22)
Neutrophil change week 0-8			
Lowered	19	79	24 (14-34)
Not lowered	26	141	18 (12-25)
Overall	49	264	19 (14-23)



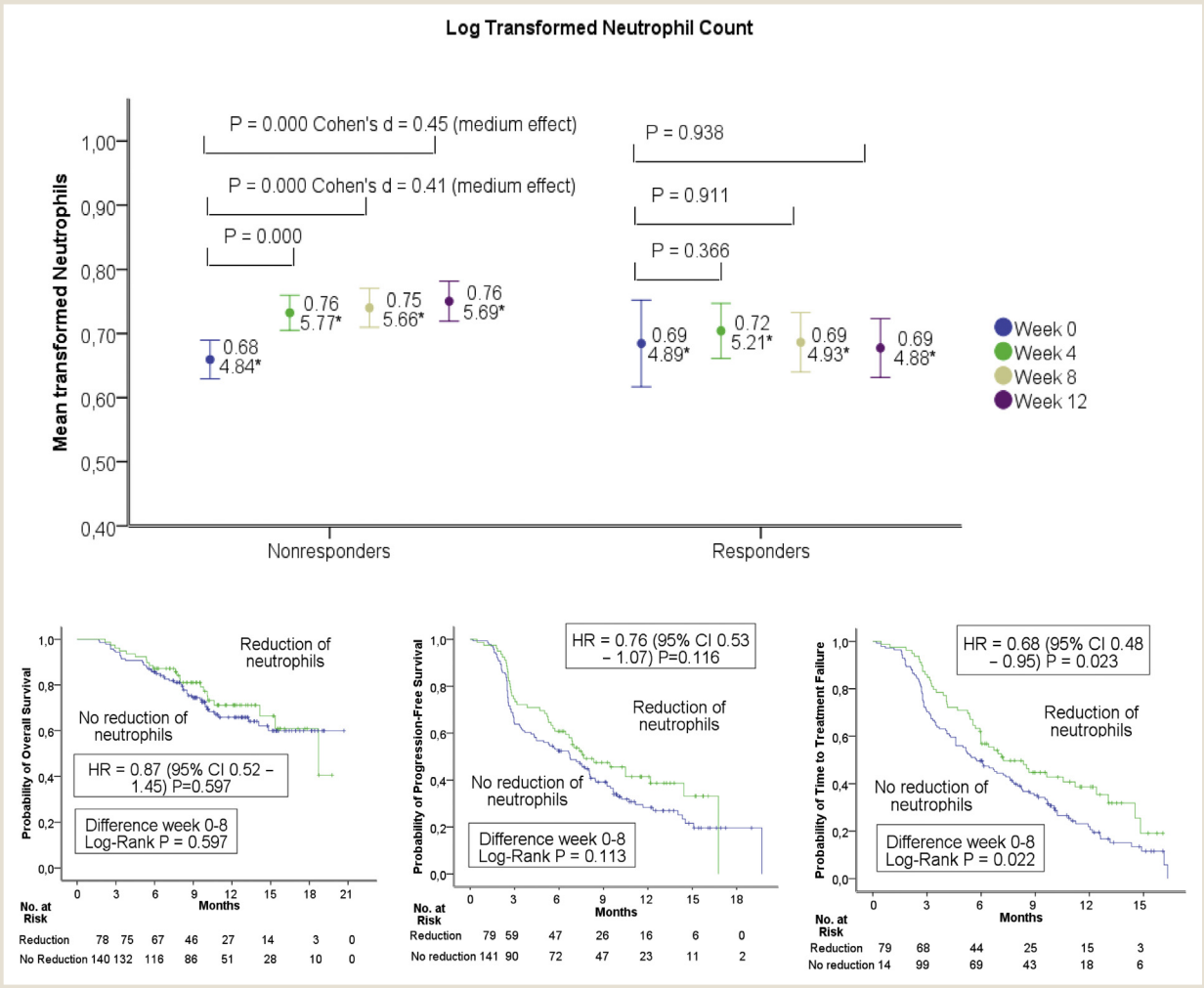
Abbreviations: CI = confidence interval; LDH = lactate dehydrogenase; MSKCC = Memorial Sloan Kettering Cancer Center; ULN = upper limit of normal; WHO = World Health Organization.

Supplemental Figure 2 Difference in Baseline Lymphocyte Level Between Responders and Non-responders. *Real Lymphocyte Count



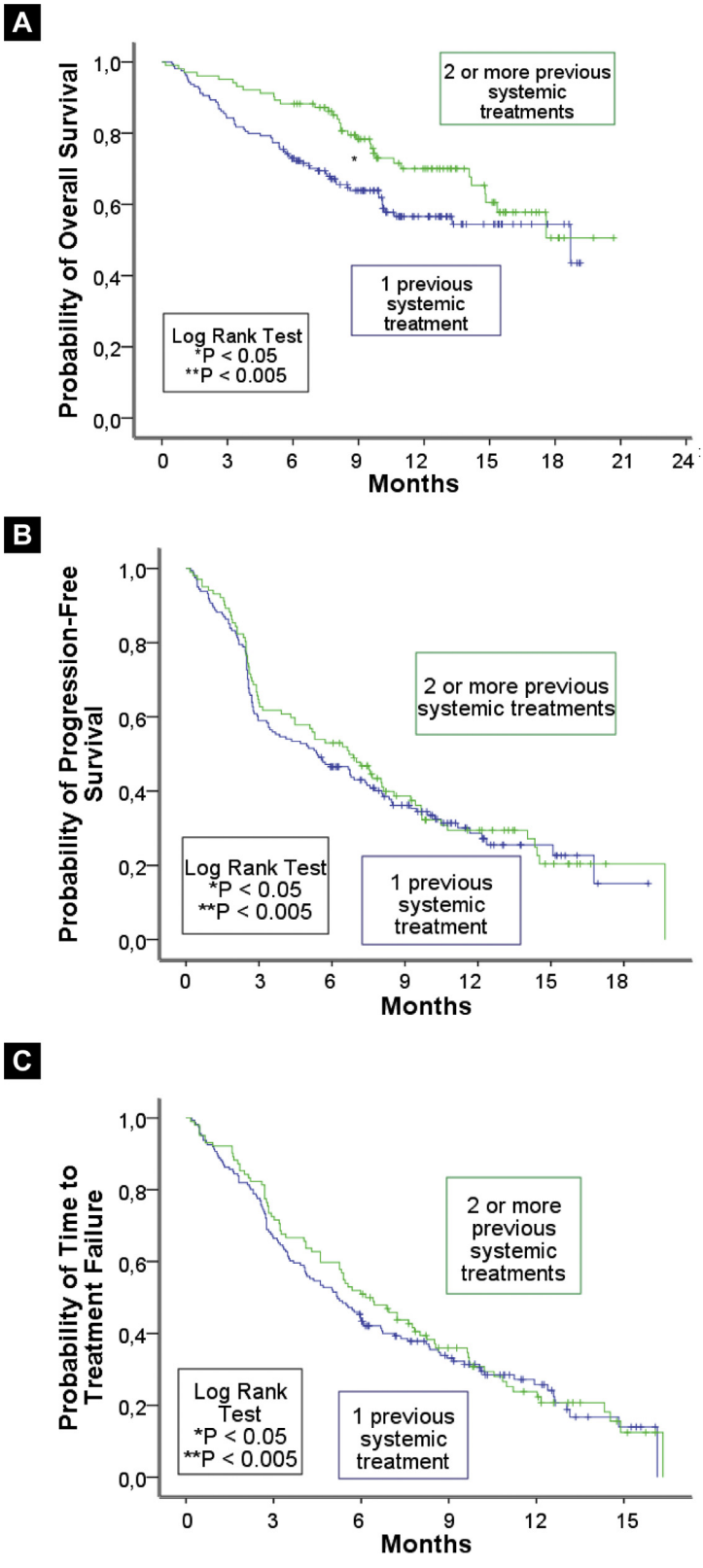
Abbreviations: CI = confidence interval; HR = hazard ratio.

Supplemental Figure 3 Difference in Baseline Neutrophil Levels Between Responders and Non-responders. *Real Neutrophil Count

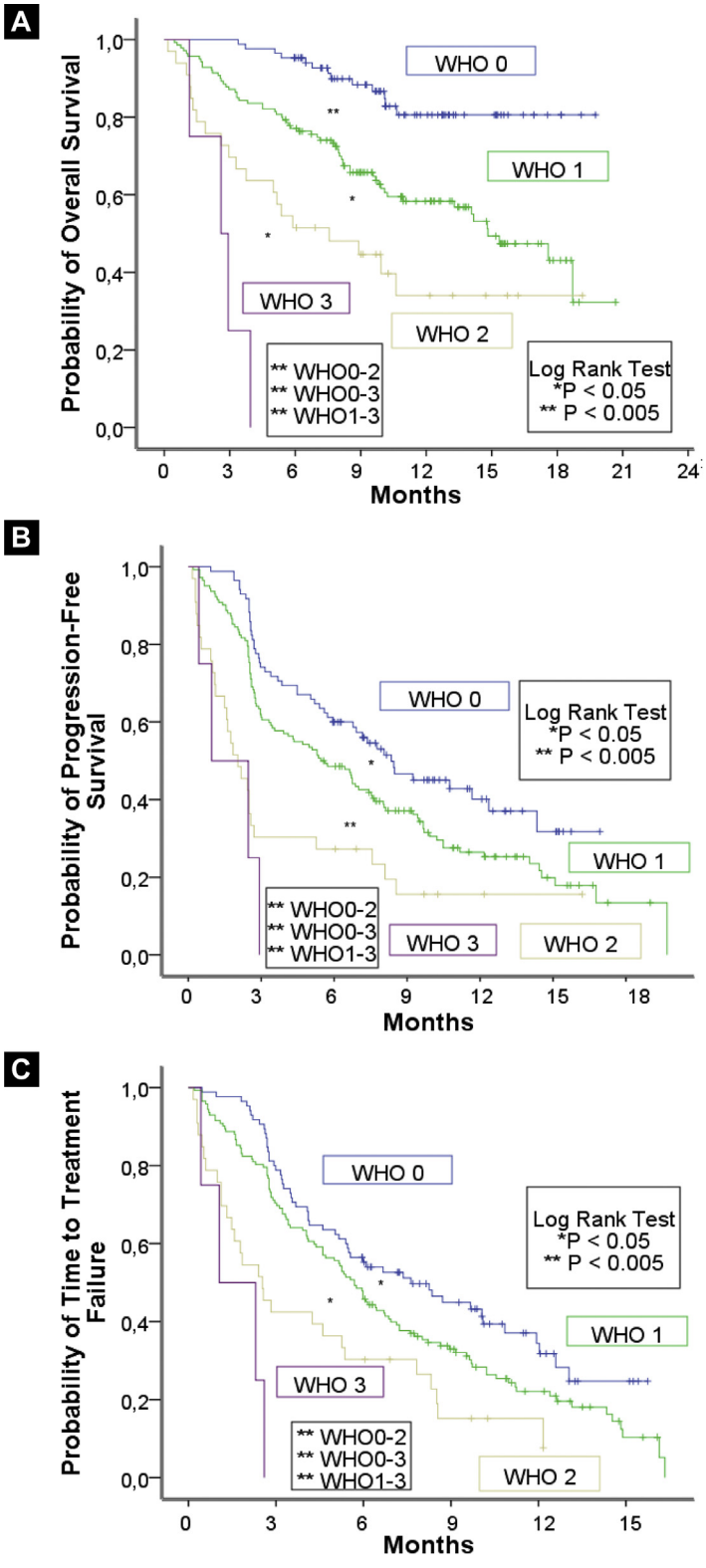


Abbreviations: CI = confidence interval; HR = hazard ratio.

Supplemental Figure 4 Overall Survival (A), Progression-free Survival (B), and Time to Treatment Failure (C) Stratified by the Number of Previous Systemic Treatments.



Supplemental Figure 5 Overall Survival (A), Progression-free Survival (B), and Time to Treatment Failure (C) Stratified by World Health Organization Performance Score



Abbreviation: WHO = World Health Organization.

Efficacy Subgroups

Risk Groups. When stratifying for MSKCC risk groups, the favorable-risk group had a median overall survival (OS) of 15.4 months (95% confidence interval [CI], not estimable). The median progression-free survival (PFS) was 8.1 months (95% CI, 4.5-11.6 months). The median time to progression (TTP) was similar to the median PFS. In the intermediate-risk group, the median OS was not estimable. The median PFS was 6.7 months (95% CI, 4.2-9.2 months). The median TTP was 6.9 months (95% CI, 4.4-9.3 months). The median time to treatment failure (TTF) was 6.4 months (95% CI, 5.1-7.7 months). In the poor-risk group, the median OS was 10.7 months (95% CI, 6.6-14.7 months). Death occurred in 49 (51%) of 97 patients. The median PFS was 4.1 months (95% CI, 1.9-6.4 months). Progression or death occurred in 77 (79%) of 97 patients. The median TTP was 5.0 months (95% CI, 2.3-7.6 months). Progression occurred in 69 (78%) of 89 patients. The median TTF was 4.1 months (95% CI, 3.0-5.2 months). Treatment failure occurred in 81 (84%) of 97 patients.

Previous Systemic Treatments. When stratifying by the number of previous systemic treatments, patients with 0 or 1 previous systemic treatment had a median OS of 18.7 months (95% CI, 6.1-31.3 months). The median PFS was 5.4 months (95% CI, 3.2-7.5 months). Progression or death occurred in 112 (69%) of 162 patients. The median TTP was 5.6 months (95% CI, 3.3-7.9 months). Progression occurred in 100 (66%) of 150 patients. The median TTF was 5.2 months (95% CI, 4.0-6.3 months). Treatment failure occurred in 120 (74%) of 162 patients. Patients with 2 or more systemic treatments had no estimable median OS. Death occurred in 33 (32%) of 102 patients. The median PFS was 6.7 months (95% CI, 4.7-8.7 months). Progression or death occurred in 73 (72%) of 102 patients. The median TTP and corresponding 95% CI were rounded the same as for PFS. Progression occurred in 70 (71%) of 99 patients. The median TTF was 6.1 months (95% CI, 4.7-7.6 months). Treatment failure occurred in 79 (77%) of 102 patients. See Supplemental Figure 4 (in the online version) for OS, PFS, and TTF treatment stratified by the number of previous systemic treatments.

Other Types of Histology. When stratifying for other types of histology, the median OS for clear-cell histology was 17.6 months (95% CI, 14.6-20.6 months). Death occurred in 88 (38%) of 234 patients. The median PFS was 5.7 months (95% CI, 4.2-7.2 months). Progression or death occurred in 165 (70%) of 236 patients. The median TTP was 6.4 months (95% CI, 5.0-7.9 months). Progression occurred in 151 (68%) of 222 patients. The median TTF was 5.6 months (95% CI, 4.8-6.3 months). Treatment failure occurred in 177 (75%) of 236 patients. In patients with other than clear-cell histology, median OS was not estimable. Death occurred in 7 (47%) of 15 patients. The median PFS was 2.6 months (95% CI, 2.4-2.7 months). Progression or death occurred in 13 (87%) of 15 patients. The median TTP and corresponding 95% CI were rounded the same as for PFS. Progression occurred in 12 (86%) of 14 patients. The median TTF was 3.3 months (95% CI, 1.4-5.1 months). Treatment failure occurred in 13 (87%) of 15 patients. Furthermore, when comparing clear-cell histology with other types of histology, there was a significant difference in PFS in favor of clear-cell histology ($P = .023$), and the same trend was visible for TTF ($P = .084$); there was no significant relationship with OS ($P = .557$).

World Health Organization (WHO) Performance Status. The WHO performance status was also noticeable in the ORR subgroup analysis. However, this is a prognostic instead of a predictive biomarker. There were significant differences between WHO performance score in OS, PFS, and TTF. Between Memorial Sloan Kettering Cancer Center (MSKCC) risk groups, there were also significant differences in OS, PFS, and TTF; however, these were less noteworthy compared with the differences in WHO performance status. In addition, OS, PFS, and TTF were also stratified by International Metastatic RCC Database Consortium (IMDC) risk groups, which presented similar to the curves of the MSKCC risk groups.

When stratifying for WHO performance score and thereby comparing WHO performance score 0 or 1, to WHO performance score 2 or 3, the median OS for WHO performance score 0 or 1 was 18.7 months (95% CI, not estimable). Death occurred in 74 (33%) of 225 patients. The median PFS was 6.7 months (95% CI, 5.3-8.2 months). Progression or death occurred in 154 (68%) of 227 patients. The median TTP was 7.0 months (95% CI, 5.4-8.6 months). Progression occurred in 141 (66%) of 214 patients. The median TTF was 6.0 months (95% CI, 5.0-6.9 months). Treatment failure occurred in 167 (74%) of 227 patients. Patients with a WHO performance score of 2 or 3 had a median OS of 5.4 months (95% CI, 1.2-9.6 months). Death occurred in 24 (65%) of 37 patients. The median PFS was 2.0 months (95% CI, 1.1-3.0 months). Progression or death occurred in 31 (84%) of 37 patients. The median TTP and 95% CI were rounded the same as for PFS. Progression occurred in 31 (84%) of 37 patients. The median TTF was 2.4 months (95% CI, 1.5-3.3 months). Treatment failure occurred in 32 (87%) of 37 patients. See Supplementary Figure 5 (in the online version) for OS, PFS, and TTF stratified by WHO performance score.

Occurrence of Adverse Events (AEs). When looking at the occurrence of severe AEs, PFS was favorable when AEs occurred ($P = .026$); OS was not ($P = .407$). When severe AEs occurred, patients had a median PFS of 8.5 months (95% CI, 4.3-12.6 months). In patients without AEs, median PFS was 5.3 months (95% CI 3.4-7.2 months). TTF was not measured because treatment was mostly discontinued when severe AEs occurred; thus, this was not a good objective for the evaluation of the relationship between efficacy and severe AEs.

Number and Sites of Metastasis. According to the subgroup analysis of Supplemental Figure 1 (in the online version), patients with more than 5 sites of metastasis had a poorer chance of having a response when compared with patients with 4 or less sites of metastasis. However, there were no significant differences in OS, PFS, and TTF when comparing these groups. When comparing 3 or less with 4 or more sites of metastasis, there were significant

differences in PFS and TTF in favor of less sites ($P = .032$ and $P = .048$, respectively). The same trend was visible for OS ($P = .110$).

In this study, patients had a higher tumor load when compared with those in the pivotal trial. When stratifying for the number of metastases and thereby comparing 4 or less sites with 5 or more, median OS for 4 or less sites of metastasis was 18.7 months (95% CI, 13.9-23.5 months). Death occurred in 76 (36%) of 211 patients. The median PFS was 5.7 months (95% CI, 3.8-7.6 months). Progression or death occurred in 145 (68%) of 213 patients. The median TTP was 6.0 months (95% CI, 4.2-7.7 months). Progression occurred in 132 (66%) of 200 patients. The median TTF was 5.8 months (95% CI, 4.7-6.8 months). Treatment failure occurred in 156 (73%) of 213 patients. In patients with 5 or more sites of metastasis, the median OS was not estimable. Death occurred in 22 (43%) of 51 patients. The median PFS was 5.4 months (95% CI, 1.2-9.6 months). Progression or death occurred in 40 (78%) of 51 patients. The median TTP was 5.4 months (95% CI, 2.9-7.9 months). Progression occurred in 38 (78%) of 49 patients. The median TTF was 5.0 months (95% CI, 3.5-6.6 months). Treatment failure occurred in 43 (84%) of 51 patients.

Brain Metastasis

Although this was not a specific objective of this study, it was noted that during nivolumab treatment, patients could get brain metastases. Among responders, 4 (8%) patients developed symptomatic brain metastases during nivolumab treatment. It is important to realize that no brain imaging was performed before starting nivolumab. Two (4%) responding patients, who had a brain scan before starting nivolumab, developed new brain metastases. Another 2 (4%) of the responding patients who already had brain metastases developed new brain metastases during nivolumab, and another (2%) responding patient who already had brain metastases had progressive disease in the brain at first. A total of 9 (18%) extracranial responders developed brain metastases during nivolumab.

In total, there were 28 patients with brain metastases before starting nivolumab, of which 5 (18%) had a response. Whether brain metastases were responding to nivolumab was not assessable because radiotherapy was applied many times (note, this was also not a predefined objective and specific numbers have not been documented during this study). Among non-responders, 9 (4%) patients developed growing brain metastases without having had imaging of the brain before starting nivolumab. It is possible that more patients developed brain metastases during nivolumab, because this was not an objective and therefore not registered properly. Two of these patients stopped nivolumab because of the growing brain metastases. Another non-responder with known brain metastases developed new brain lesions.

Albumin, hemoglobin, C-reactive protein, erythrocyte sedimentation rate, thrombocytes, and lowering of lactate dehydrogenase (LDH) were also analyzed but did not reveal significant results (data not shown). Most intriguing were the eosinophil, lymphocyte, and neutrophil changes between week 0 and 8 (see main text).

A reduction in LDH level compared with no reduction of LDH in relation with overall survival (OS), progression-free survival (PFS), and time to treatment failure (TTF) did not result in significant results. The difference between LDH week 0 and weeks 4, 8, and 12 was significant in non-responders (see main text) ($P = .004$, $P = .018$, and $P = .005$, respectively), but not in responders ($P = .584$, $P = .541$, and $P = .249$, respectively). There was no significant difference in baseline LDH level between responders and non-responders. However, a normal baseline LDH level was related to an improved OS, PFS, and TTF when compared with an elevated baseline LDH level ($P = .000$, $P = .000$, and $P = .000$, respectively). The median OS was not estimable versus 9.7 months (95% confidence interval [CI], 7.5-11.9 months), the median PFS was 7.6 months (95% CI, 5.8-9.3 months) versus 2.8 months (95% CI, 2.4-3.2 months), and the median TTF was 6.7 months (95% CI, 5.2-8.2 months) versus 2.9 months (95% CI, 2.1-3.6 months), respectively.

The differences among eosinophils between week 0 and weeks 4, 8, and 12 were significant for both responders and non-responders (see main text). However, it was not possible to make a normal distribution of eosinophil counts; therefore, there are no measurements of effect size.

The differences between week 0 and weeks 4, 8, and 12 in lymphocyte count were significant for responders but not for non-responders (see main text). To make parametric testing possible, the lymphocytes were square-root transformed. Weeks 0, 4, 8, and 12 were thereafter all normally distributed. The largest effect was an elevation of lymphocytes in responders measured between week 0 and week 8. With a paired *t* test, the Cohen d was 0.68, meaning a large effect ($P = .000$) according to Cohen. Between week 0 and 4, the elevation of lymphocytes in responders had a Cohen d of 0.57 ($P = .000$), and the Cohen d between week 0 and 12 was 0.45 ($P = .006$), representing both a medium effect (see Supplemental Figure 2 in the online version.

Notably, there was no significant difference in baseline lymphocyte count between responders and non-responders. There was a significant benefit for normal and elevated baseline lymphocyte count in PFS when compared with lowered baseline lymphocyte count ($P = .038$). The median PFS was 7.0 months (95% CI, 5.4-8.6 months) versus 3.2 months (95% CI, 1.2-5.1 months), respectively. However, no relation with OS and TTF was found.

The neutrophil count was significantly different for non-responders between week 0 and weeks 4, 8, and 12 (see main text). This same level of significance was reached when log-transformed to achieve a normal distribution. The neutrophil count of week 4 was not normally distributed by log transformation; thus, the effect size between weeks 0 and 4 could not be measured. For responders, there was no significant difference between week 0 and weeks 4, 8, and 12 ($P = .366$, $P = .976$, and $P = .963$, respectively, for the untransformed data). The greatest effect was measured between neutrophil level week 0 and week 12 in non-responders: Cohen d was 0.45, meaning a medium effect. Between week 0 and 8 there was also a medium effect (Cohen d of 0.41) (see Supplemental Figure 3 in the online version).

There was no significant difference in baseline neutrophil count between responders and non-responders. A significant benefit was found for the lowered and normal baseline neutrophil count in relation to OS when compared with elevated baseline neutrophil count ($P = .040$). The median OS was 18.7 months (95% CI, 14.0-23.4 months) versus 10.1 months (95% CI, not estimable), respectively. There was no significant relation with PFS and TTF.

Subsequent Therapy

Among 223 registered patients in whom subsequent therapies were registered, 74 (33%) received subsequent therapy, and 7 (3%) other patients might also continue with subsequent therapy. The therapeutic agents used after treatment with nivolumab were cabozantinib (19%; 43 patients), everolimus (9%; 20 patients), sunitinib (3%; 7 patients), pazopanib (3%; 7 patients), axitinib (2%; 4 patients), lenvatinib in combination with everolimus (>1%; 2 patients), bevacizumab (>1%; 1 patient), and bevacizumab in combination with interferon (>1%; 1 patient).