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Original Research

Correlation between nivolumab exposure and treatment outcomes in non–small-cell lung cancer

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Abstract ***Introduction:*** Nivolumab treatment is subject to large interpatient variability in both efficacy and toxicity, which may partly be explained by differences in nivolumab exposure. Exposure–response relationships in regular healthcare have not been extensively investigated for nivolumab. Therefore, we aimed to identify possible exposure–response relationships in nivolumab-treated patients with non–small-cell lung cancer (NSCLC).
Methods: Patients with NSCLC who started second-line nivolumab therapy (3 mg/kg Q2W) between May 5th 2016 and August 1st 2017, and from whom serial blood samples, toxicity data and outcome data were prospectively collected, were included. Follow-up was carried out until November 1st 2017. Patients were classified according to the best overall response (BOR) based on the Response Evaluation Criteria in Solid Tumours, v1.1, and toxicities according to the Common Terminology Criteria for Adverse Events. Nivolumab trough concentrations were measured after 2, 4 and 10 weeks of treatment, excluding dose delays, and calculated geometric means were tested versus BOR or toxicity using analysis of variance and an independent samples *t*-test, respectively. Overall survival (OS) and progression-free survival were compared between high and low trough concentration groups.

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Results: Seventy-six patients were evaluable for analyses. Responders (n = 15) had higher mean trough concentrations than patients with progression (n = 33): 47% higher after 2 weeks (p = 0.001), 53% higher after 4 weeks (p = 0.008) and 73% higher after 10 weeks (p = 0.002). Higher trough concentrations were associated with longer OS (p = 0.001).
Conclusions: This study shows that patients with NSCLC with a response to nivolumab had a higher nivolumab exposure than patients with progression, indicating a potential exposure–response relationship. Further clinical research should focus on clarifying these exposure–response relationships.

1. Introduction

Nivolumab is a human immunoglobin G4 monoclonal antibody directed against programmed death 1 (PD-1) protein, reinvigorating intratumoural T-cells, which often have become inactivated in a T-cell costimulation-deprived microenvironment [1]. Nivolumab is currently approved for the treatment of various solid and haematological malignancies.

PD-1 receptor occupancy on circulating CD3+ T-cells in nivolumab-treated melanoma patients has been demonstrated to be saturated at doses above 0.3 mg/kg, as patients receiving higher doses did not achieve a higher PD-1 receptor occupancy. However, response rates in these patients were higher in doses >0.3 mg/kg [2,3], indicating the presence of unexplored mechanisms which determine response to nivolumab treatment. Comparable results are reported for nivolumab-treated patients with NSCLC: response rates in patients dosed at 3 mg/kg Q2W were higher than those dosed at 1 mg/kg Q2W (24% versus 3%). However, no increase in response rates was observed in patients receiving 10 mg/kg Q2W compared with 3 mg/kg Q2W [2,3]. Moreover, the occurrence of serious adverse events did not increase in patients who received nivolumab doses of 1.0 mg/kg or higher [3]. Therefore, the dose of 3 mg/kg Q2W was used in consecutive phase 3 trials [4,5]. As a consequence, subtle exposure–response relationships in NSCLC patients receiving the nivolumab dose of 3 mg/kg may have been overlooked.

So far, exposure–response relationships in nivolumab-treated patients have not been reported [6], whereas various other monoclonal antibodies used for the treatment of solid tumours and haematologic malignancies have shown exposure–response relationships [7–11]. For example, breast cancer patients treated with trastuzumab with an exposure in the lowest quartile after cycle 1 had 8-month shorter median overall survival (OS) than patients in other quartiles, without a difference in the occurrence of toxicities. Patients with colorectal cancer with a below-median cetuximab exposure experienced significantly shorter median progression-free survival (PFS) than other patients (3.3 versus 7.8 months). Furthermore, in patients with advanced melanoma, ipilimumab 10 mg/kg resulted in significantly longer median OS than ipilimumab 3 mg/kg (15.7 versus 11.5 months) [12]. For the first time, we studied exposure–response relationships in standard of care nivolumab-treated patients with NSCLC, treated with a dosing regimen of 3 mg/kg Q2W.

2. Materials and methods

2.1. Design

Patients with stage IV NSCLC who started nivolumab treatment between May 5th 2016 and August 1st 2017 at the Erasmus MC Cancer Institute were included prospectively in this study (Dutch Trial Registry number NTR7015), allowing for serial sampling during standard of care nivolumab treatment. Data cut-off was at November 1st 2017. Blood was drawn every 2 weeks before nivolumab infusion to measure nivolumab trough concentrations (i.e. concentration immediately before the following infusion). Patient characteristics and clinical data were included from the hospital's electronic patient record system. Best overall response (BOR) was assessed according to the Response Evaluation Criteria in Solid Tumours (RECIST) v1.1, with a minimal follow-up time of 90 days [13]. If treatment was discontinued before 90 days because of rapid progression or death, BOR was classified as progressive disease (PD). A minimum duration of 90 days for stable disease (SD) was necessary. Confirmation of partial response (PR) or complete response was not required for the best response determination. In patients treated beyond radiologic progression, subsequent response assessments accounted for BOR. Adverse events were registered from the start of treatment until the end of follow-up according to the National Cancer Institute Common Terminology Criteria for Adverse Events, v4.03.

2.2. Nivolumab serum measurements

Before every nivolumab infusion, 4 mL of serum was obtained, of which 5 μL was used to measure

nivolumab serum concentrations. All nivolumab concentrations were measured with an in-house developed and validated enzyme-linked immunosorbent assay [14]. Measurements were performed as follows: 96-well EIA/RIA microtiter plates (Corning, NY, USA) were coated overnight at 4 °C with 100 µL/well of 0.1 µg/mL of capture antigen (recombinant human PD-1 Fc chimera, R&D, Minneapolis, MN, USA), diluted in phosphate-buffered solution (PBS) (BioWhittaker Inc, Walkersville, MD, USA), pH 7.4. The plates were washed three times in washing buffer (0.05% Tween 20 in PBS, pH 7.4) and incubated for 1 h at room temperature (RT) with 300 µL/well of blocking solution (1% BSA/0.05% Tween 20 in PBS, pH 7.4). After three washes, nivolumab calibration standards, ranging from 25 to 0.20 ng/mL, and serum samples, 1:4000 diluted in blocking solution, were added in duplicate to the wells, and subsequently, the plates were incubated for 2 h at RT on a shaker set at 300 rpm. After three additional washes, 100 µl/well of 0.2 µg/mL of detection antibody (Human IgG4-HRP, Bio-Rad, Hercules, CA, USA), diluted in the blocking solution, was added to the plates, followed by incubation for 2 h at RT on a shaker. Subsequently, the plates were washed three times, and 100 µl/well of tetramethylbenzidine peroxidase substrate was added. The plates were incubated for 15 min in the dark, and the reaction was stopped with 2 M H_2SO_4. Results were read using VersaMax Tunable microplate reader (Molecular Devices) at an optical density of 450 nm, corrected at wavelength of 570 nm. Results were calculated by averaging all duplicate readings and using a standard curve, generated by a 5 parameter logistic curve fit.

2.3. Statistics and data analysis

Patients were divided according to BOR in PD, SD and PR and according to toxicity (occurrence of grade ≥III or grade ≤II adverse events). Trough concentrations were compared on the log scale at week 2, 4 and 10 between BOR groups with analysis of variance and between toxicity groups with an independent samples t-test. Patients with dose delays until the time point of analysis were excluded for those particular—and following—time points. If significant, a *post hoc* analysis with Tukey honest significant difference test was performed between BOR groups. The assumption of equal variances in log-transformed trough concentrations between groups was assessed with Levene's test. A log rank test was performed to assess potential differences in OS and PFS between the groups with 50% lowest trough concentrations (low exposure) and 50% highest trough concentrations (high exposure). Patients dying without progression were censored. Data collection and statistical analysis were performed using R v3.3.1 and IBM SPSS Statistics, v24 (Chicago, IL). A two-sided p value < 0.05 was considered significant.

3. Results

Of 84 patients, 76 were evaluable (see Table 1) because four patients were non-evaluable according to RECIST and four patients had no follow-up blood samples available as their treatment was discontinued after the first cycle (three patients because of rapid clinical deterioration and one patient because of grade III skin toxicity). According to BOR, 33, 28 and 15 patients had PD, SD and PR, respectively. Grade ≥III toxicities occurred in 15 patients. Median follow-up time was 246 days (interquartile range 127–379 days). The courses of median nivolumab trough concentrations per response group until 20 weeks after treatment start are shown in Fig. 1. Comparisons of median trough levels and the number of evaluable patients per response group at week 2, 4 and 10 are shown in Fig. 2. In Fig. 3a and b, Kaplan–Meier curves are shown for OS and PFS per group of trough concentration, whereas median trough concentrations and the number of evaluable patients per toxicity group are shown in Fig. 4. For each time point, geometric mean trough concentrations were significantly different between response groups: at week 2: $p = 0.001$; at week 4: $p = 0.01$; at week 10: $p = 0.002$. *Post hoc* comparisons showed that at week 2, PR patients (27.4 µg/mL, 95% CI: 22.3–33.6 µg/mL) had 47% (95% CI: 34–61%) higher geometric mean trough concentrations than PD patients (18.7 µg/mL, 95% CI: 16.7–20.9 µg/mL; $p = 0.001$) and 30% (95% CI:

Table 1
Patient characteristics.

Characteristic	Total number of patients (n = 76)	Non-responders (n = 33)	Partial responders (n = 15)
Gender			
Male	46 (61%)	21 (64%)	6 (40%)
Female	30 (40%)	12 (36%)	9 (60%)
Age at start (years)			
Mean (± standard deviation)	63 (±8.9)	60 (±9.7)	65 (±6.6)
WHO performance status			
0	17 (22%)	5 (15%)	3 (20%)
1	39 (51%)	20 (61%)	10 (67%)
2	1 (1%)	1 (3%)	0 (0%)
Unknown	19 (25%)	7 (21%)	2 (13%)
Primary lung tumour			
Adenocarcinoma	50 (66%)	22 (67%)	12 (80%)
Squamous cell carcinoma	23 (30%)	10 (30%)	2 (13%)
Large cell carcinoma	3 (4%)	1 (3%)	1 (7%)
Number of pretreatment lines[a]			
1	60 (79%)	23 (70%)	14 (93%)
2	14 (18%)	10 (30%)	0 (0%)
3	2 (3%)	0 (0%)	1 (7%)

WHO, World Health Organization.
[a] All patients with pretreatment received a platinum-containing regimen.

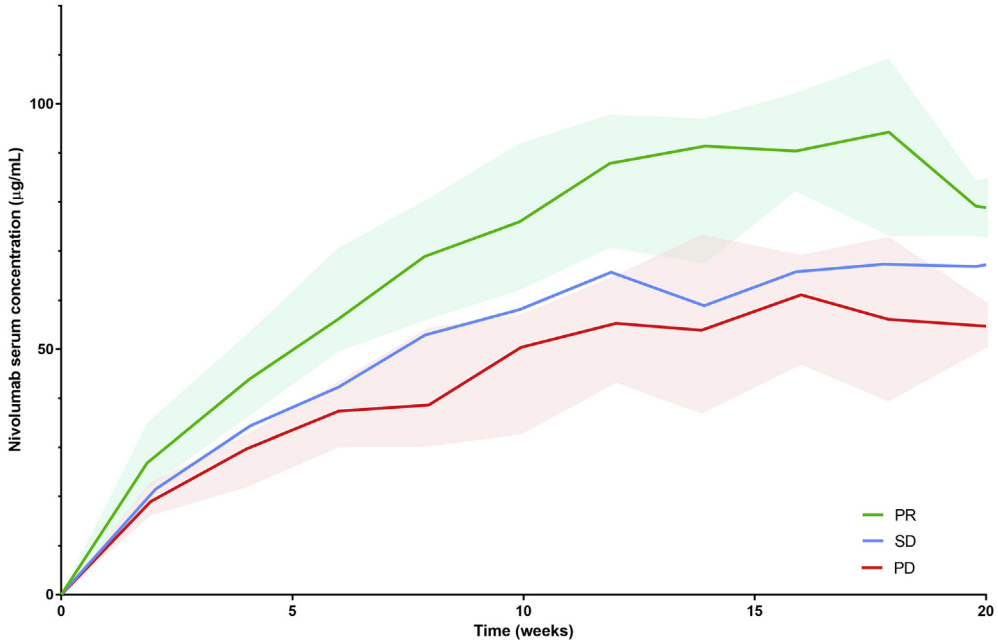


Fig. 1. **Median nivolumab trough concentrations.** Nivolumab trough concentrations for each response category, measured before every nivolumab infusion and 2 weeks after a previous infusion. Lines represent median values for PR (green), SD (blue) and PD (red), respectively. The shaded area represents interquartile ranges for partial responders (green) and patients with progressive disease (red). PR, partial response; SD, stable disease; PD, progressive disease. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

20–42%) higher trough concentrations than SD patients (21.0 µg/mL, 95% CI: 18.6–23.7 µg/mL; p = 0.034). At week 4, PR patients (46.2 µg/mL, 95% CI: 37.4–57.0 µg/mL) had 53% (95% CI: 50–57%) higher trough concentrations than PD patients (30.2 µg/mL, 95% CI: 25.0–36.4 µg/mL; p = 0.008) and 40% (95% CI: 32–48%) higher trough concentrations than SD patients (33.0 µg/mL, 95% CI: 28.3–38.5 µg/mL; p = 0.047). At week 10, PR patients (79.4 µg/mL, 95% CI: 60.7–103.8 µg/mL) had 73% (95% CI: 71–76%) higher trough concentrations than PD patients (45.8 µg/mL, 95% CI: 35.6–58.9 µg/mL; p = 0.002), whereas trough concentrations in SD patients were 36% (95% CI: 21–54%) higher than those in PD patients (62.5 µg/mL, 95% CI: 54.9–71.3 µg/mL; p = 0.048). The high-exposure group experienced significant longer OS (median: not reached versus 306 days; p = 0.001), whereas no significant difference was found for PFS (median: 189 versus 77 days; p = 0.061).

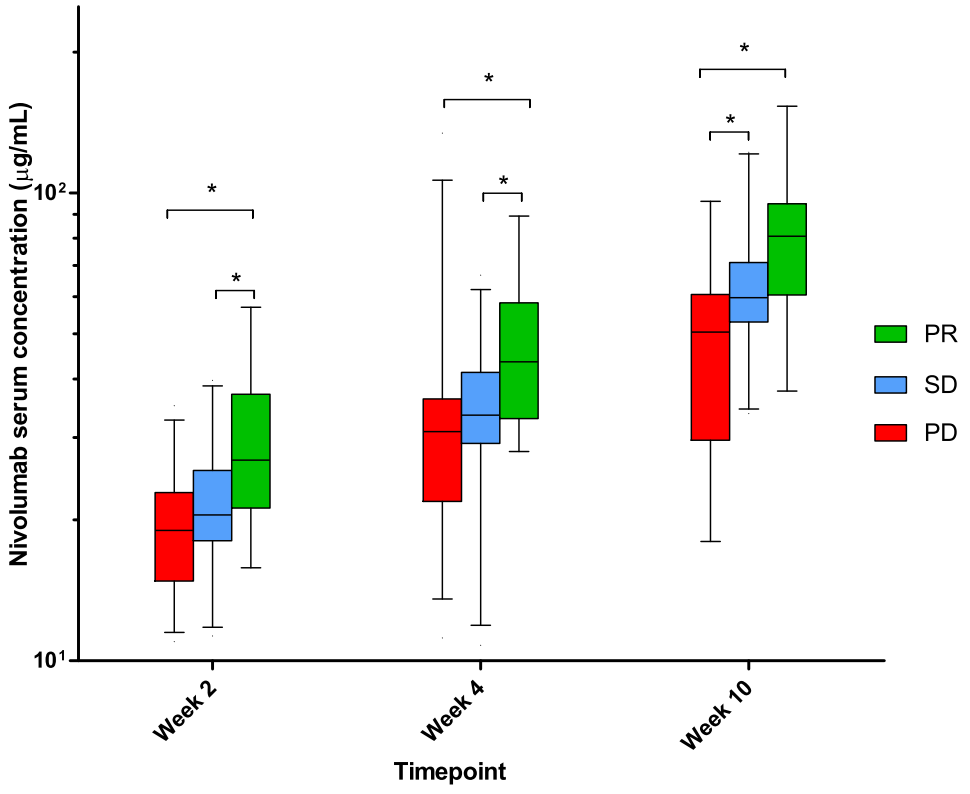
No difference in exposure was found when comparing patients with and without grade ≥III toxicity during all time points: 4% (95% CI: 3–5%) difference at week 2 (p = 0.732), 21% (95% CI: 20–23%) difference at week 4 (p = 0.216) and 20% (95% CI: 12–32%) difference at week 10 (p = 0.413). Assumption of equal variances between BOR groups and between toxicity groups was met at each investigated time point.

Only after 4 weeks of treatment, a difference in exposure between men and women was found (30.4 µg/mL versus 41.3 µg/mL; p = 0.005).

4. Discussion

In this study, we aimed to assess the relationship between nivolumab exposure and clinical outcomes in patients with NSCLC. We demonstrated for the first time that patients, treated with an equivalent dose per kg and with an objective radiographical response to nivolumab therapy, have a significantly higher exposure than non-responders at all the time points measured (i.e. after 2, 4 and 10 weeks of treatment). The high-exposure group experienced longer OS, whereas no difference was found for PFS. No association was found between the occurrence of grade ≥III adverse events and drug exposure, which is in line with earlier phase I studies, where no maximum tolerated dose could be defined for nivolumab in the dose range of 0.3–10 mg/kg [15].

These observations might reflect a true exposure–response relationship for nivolumab. On the other hand, a target concentration of 10 µg/mL was sufficient in an *ex vivo* model for reaching >90% of the maximum achievable receptor occupancy [16], which is already reached after the first cycle in all treatment groups. Furthermore, it has recently been suggested that the exposure–response relationship for immune checkpoint inhibitors is confounded by the catabolic state due to cancer cachexia, which would lead to lower nivolumab concentrations because of accelerated IgG breakdown and would shorten survival [17]. In that study, however, the exposure–response relationship could not be explained by cachexia alone, indicating that other



BOR	N	N	N
PD	29	27	15
SD	24[a]	25	20
PR	14	14	11

Fig. 2. **Nivolumab trough concentrations per time point according to BOR.** Nivolumab trough concentrations per response category per time point. Red, blue and green boxes represent the median and interquartile ranges for the PD, SD and PR groups, respectively. Whiskers show 5–95% percentile. ∗Indicates significant difference ($p < 0.05$); [a]One missing sample. PR, partial response; SD, stable disease; PD, progressive disease. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

factors than only peripheral PD-1 receptor occupancy are likely involved in a response to treatment too, as has been suggested earlier [2]. Moreover, our primary endpoint (i.e. best overall response) is less likely to be affected by cachexia than OS is. Currently available evidence is, therefore, not sufficient to rule out an exposure-dependent anticancer effect of nivolumab, and it remains vital to further elucidate the relationship between exposure and response. Additionally, an increasing response rate until a nivolumab dose of 3.0 mg/kg Q2W in patients with NSCLC [2,3] supports a possible exposure–response relationship in patient groups receiving 3.0 mg/kg Q2W.

If exposure appears to determine response (at least partially), we hypothesise that PD and SD patients would have had a better response if they had reached higher systemic exposure at an earlier moment because nivolumab trough concentrations are higher in PR patients in an early phase of treatment. To achieve this, a loading dose could be considered, for example, by doubling the first dose of nivolumab. The long terminal half-life of the nivolumab IgG antibody, leading to a long time until steady state is reached, supports such a loading dose, which is applied for many other therapeutic IgG antibodies too [18]. Many factors have been demonstrated or are thought to influence the pharmacokinetics of monoclonal antibodies. Along with cachexia, the influence of other parameters on monoclonal antibody exposure needs to be quantified; the body weight has been correlated with the clearance of monoclonal antibodies [19]. And clearance itself is associated with OS in pembrolizumab-treated patients [17]. Also, it is thought that endothelial wall inflammation may influence the distribution of monoclonal antibodies [20]. Furthermore, (epi)genetic variation in the neonatal Fc-receptor may influence pharmacokinetics of certain antibodies [21]. Target-mediated clearance and thus PD-1 expression may affect exposure too [22,23]. Furthermore, the formation of anti-drug antibodies (ADAs) influences pharmacokinetics of administered antibodies [21]. Although a negligible effect was seen on efficacy and no effect was seen on clearance, 12.7% of nivolumab-treated patients experience ADA formation [24].

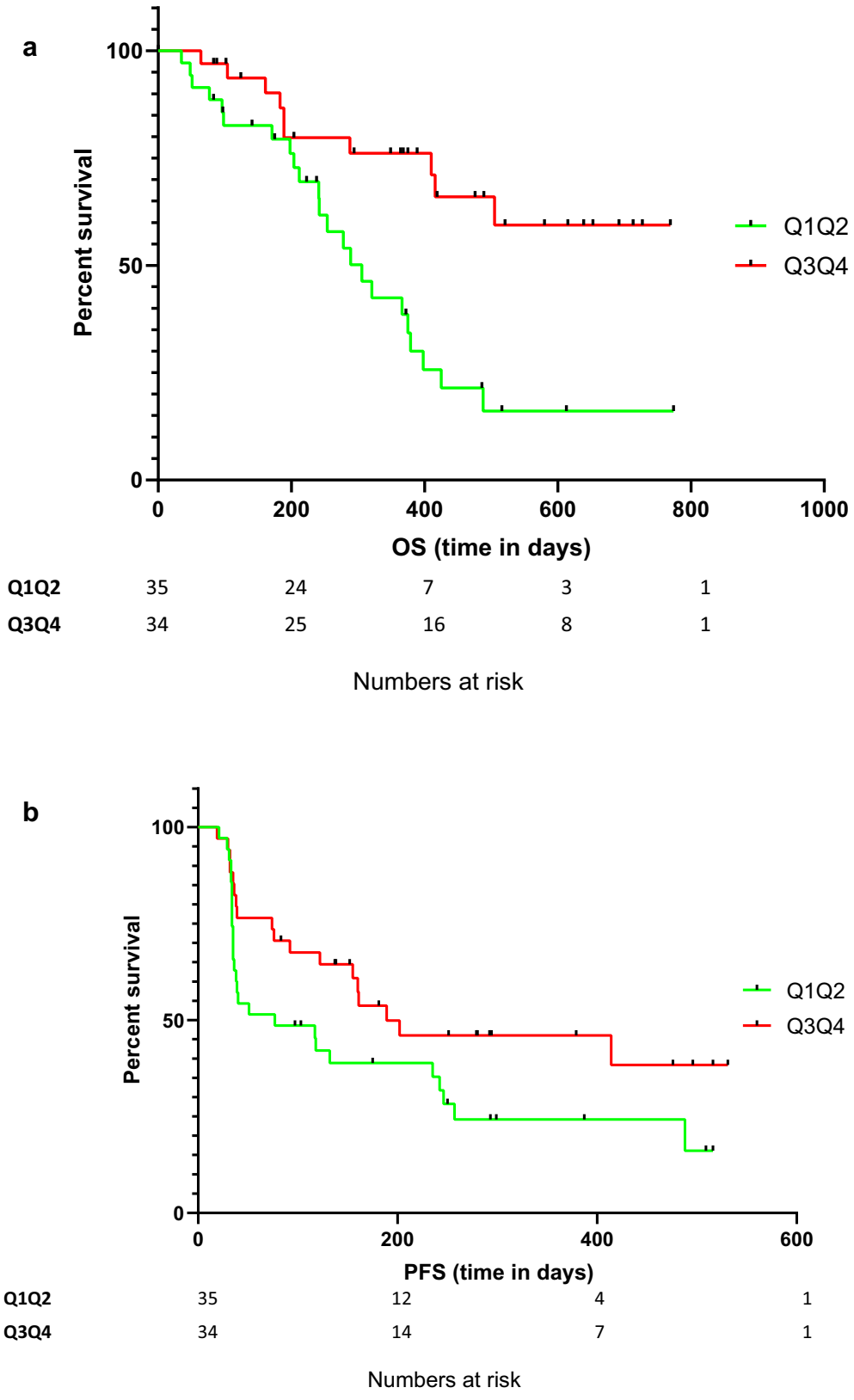
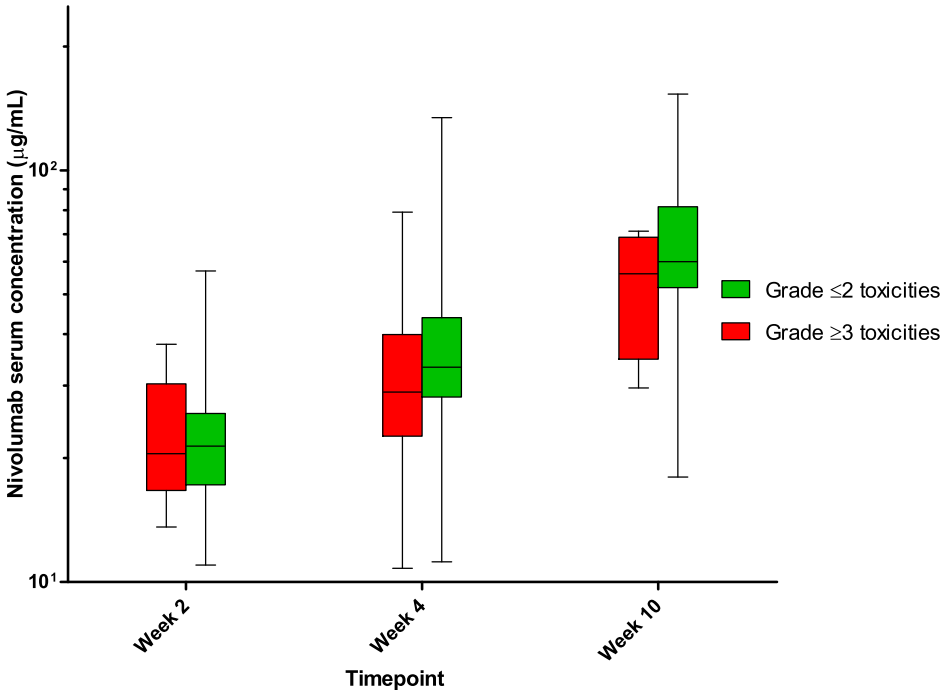


Fig. 3. **OS and PFS versus nivolumab trough concentrations.** Kaplan–Meier curve for overall survival (a) and progression-free survival (b) stratified for the groups with 50% lowest trough concentrations (Q1Q2) and 50% highest trough concentrations (Q3Q4). OS, overall survival; PFS, progression-free survival.



Toxicity	N	N	N
Grade 3 or higher	11	10	4
Grade 2 or lower	56	56	42

Fig. 4. **Nivolumab trough concentrations per time point according to toxicity.** Nivolumab trough concentrations per toxicity category per time point. Green and red boxes represent the median and interquartile ranges for the grade ≤II toxicity and grade ≥III toxicity groups, respectively. Whiskers show 5–95% percentile. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

In our analysis, the high-exposure group experienced longer OS, whereas no difference was found for PFS. This may indicate that trough concentrations are affected by mechanisms influencing OS but less PFS, such as cachexia. However, subsequent treatment lines after nivolumab may also influence OS, whereas PFS is not affected.

Male patients experienced lower exposure only after 4 weeks of treatment; this is in line with earlier findings, reporting higher clearance in men [17,25].

Serial sampling of blood allowed us to include time points before a radiologic assessment and therefore to study a high number of patients treated with an equivalent dose per kg. The prospective character of this study, the inclusion of a uniform cohort treated with a similar nivolumab dosage, the distribution of the response groups comparable to earlier reported trials and intensive sampling prior nivolumab administration provide a solid background for interpretation of results. Intensive measurements of C_{trough} levels are—to our opinion—excellent means to study exposure–response relationship because it is relatively convenient for patients and it is the most informative pharmacokinetic sample to quantify exposure in a single pharmacokinetic sample strategy. Moreover, minimum and median follow-up time (3 and 8.1 months, respectively) well exceeded median time to response (2.1–2.2 months) [4,5] and toxicity, that generally occurs within 3 months [26]. Therefore, only less data on the clinical end-points is lacking in this analysis. In a real-world setting, physicians occasionally decide for treatment beyond progression, two patients had 'pseudo-progression'. Both patients eventually achieved PR during nivolumab therapy and were, therefore, classified accordingly. Regarding the PK data, one should notice that patients with serious adverse events or PD were excluded from analysis at week 4 and 10 relatively more frequent because of interruption or discontinuation of treatment, respectively, which may lead to a higher decrease of included patients at later time points in those subgroups. Some caution when interpreting the data should be taken because potential factors associated with treatment outcomes, such as the tumour load, the occurrence of pre-existent cachexia or clearance [17] and their influence on exposure, are not included in this analysis. Also, the included number of patients in this analysis is relatively low. We did not perform a multivariable analysis because of potential sparse-data bias. Although it has been noted that exposure–response relationships in nivolumab-treated patients may be biased by decreased clearance in responding patients, this finding is shown to be of less relevance in an early stage of

treatment [27]. This emphasises the need for further research following our results, despite earlier analysis showing a relatively flat exposure–response relationship over various nivolumab doses [28].

This is the first study showing an exposure–response relation for nivolumab. We argue that further clarification of exposure–response relationships and its covariates in patients treated with nivolumab is highly warranted, and new dosing strategies or combination therapies aiming at increasing the dosage should be explored. In particular, as toxicity does not increase with a higher systemic exposure, future nivolumab dosing adjustments based on exposure may improve treatment outcomes.

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Conflict of interest statement

A.A.M.V. has a consulting or advisory role at BMS. J.G.J.V.A. has a consulting or advisory role at BMS and has patents pending for analysis of biomarkers for immunotherapy. R.H.J.M. has patents pending for analysis of biomarkers for immunotherapy. All remaining authors declare no potential conflicts of interest.

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