

Prospective real-world study on the pharmacokinetics of pembrolizumab in patients with solid tumors

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ABSTRACT

Background Dosing schemes of pembrolizumab (anti-programmed cell death protein 1 monoclonal antibody) are solely based on pharmacokinetic (PK) modelling derived from phase I–III trials. The current study aimed to determine factors affecting PK and its relationship with clinical outcome in the real-world setting.

Methods Advanced-stage cancer patients, who were treated with pembrolizumab monotherapy (2 mg/kg Q3W or 200 mg flat Q3W), were prospectively included for serial sampling to obtain trough concentrations. A PK model was generated, covariate effects assessed and internally validated by a bootstrap procedure. PK parameters were related to overall survival (OS) and the occurrence of immune-related adverse events (irAEs).

Results 588 serum samples derived from 122 patients with (non-)small-cell lung cancer (NSCLC), malignant pleural mesothelioma (MPM), melanoma and urothelial cell cancer (UCC) were analyzed. Median follow-up was 2.2 years. A one-compartment PK model was generated: body surface area (BSA) and serum albumin had a significant effect on drug clearance (CL; covariate estimate 1.46 and −1.43, respectively), and serum lactate dehydrogenase (LDH) on the distribution volume (V_d ; 0.34). A significant inverse CL–OS relationship was determined for NSCLC (HR:1.69; 95%CI1.07–2.68; $p=0.024$) and MPM (HR:3.29; 95% CI 1.08 to 10.09; $p=0.037$), after correction for prognostic factors, which could not be confirmed for melanoma ($p=0.22$) or UCC ($p=0.34$). No relationship could be determined between CL and grade ≥ 3 irAEs ($p=0.70$).

Conclusions High interpatient variability of pembrolizumab PK is determined by BSA and serum albumin (on CL) and LDH (on V_d). A strong inverse CL–OS relationship was demonstrated for NSCLC and MPM, which could not be observed for melanoma and UCC. The findings suggest that personalized dosing should be prospectively explored.

BACKGROUND

Monoclonal antibodies that inhibit the programmed cell death protein 1 (PD-1) receptor or its ligand (PD-L1) are standard of care for many types of cancer.^{1 2} The clinical development of immune checkpoint

inhibitors (ICIs) differed from cytotoxic drugs in oncology. While traditional drugs generally have a small therapeutic range with a profound exposure–toxicity relationship, ICIs are relatively well tolerated.³ Pembrolizumab, a humanized monoclonal antibody directed against the PD-1 receptor, is approved for several solid tumors at a dose of 2 mg/kg every 3 weeks (Q3W), a flat dose of 200 mg (Q3W), and more recently as a 400 mg flat dose every 6 weeks (Q6W).⁴ While the weight-based dosing schemes were based on phase I/II trials, investigating the efficacy and dose-limiting toxicities (DLTs) of pembrolizumab,^{5–8} the approval of the flat dosing schemes was solely based on pharmacokinetic (PK) modeling.^{9–11} An interim analysis in metastatic melanoma patients who received 400 mg dose Q6W was encouraging, with a similar objective response rate (ORR) and safety profile compared with the Q3W regimens (2 mg/kg or 200 mg).¹² As maximum receptor occupancy is reached with doses >1 mg/kg Q3W,¹³ no efficacy of lower dosing schemes has been expected.¹⁴ However, dose-finding studies for ICIs could generally not identify a dose–response relationship for dosing schemes with pembrolizumab >0.1 mg/kg Q3W.^{5 7 8 15–17} Interestingly, an exposure–response relationship was found across tumor types, which was hypothesized to be the result of the interpatient variability of drug clearance (CL).^{18–20} Indeed, it was observed that patients with a faster drug CL had impaired survival outcomes, irrespective of pembrolizumab dosing.²⁰

Population PK modeling of PD-1 ICIs has previously been performed on data from phase I–III trials^{21–24} and for nivolumab also on real-world data.¹⁹ These previous studies described the pembrolizumab PK

using two-compartment models with linear elimination or non-linear time-varying elimination.²³ Population PK parameters were similar among different PD-1 agents. Importantly, as PK may differ between patient populations (eg, due to differences in tumor type or patient characteristics), it is highly relevant to study these covariate effects by PK modeling of pembrolizumab in the real-world setting. For instance, serum albumin levels at baseline have been consistently reported to be associated with a lower CL of pembrolizumab.^{21,24}

Therefore, serial blood sampling was performed in two hospitals in The Netherlands as part of a large prospective study in the real-world setting. The current analysis aims to (1) determine patient parameters influencing pembrolizumab PK and (2) describe the relationship between systemic pembrolizumab CL and clinical outcome (overall survival (OS), progression-free survival (PFS) and best overall response (BOR)) in patients with advanced-stage cancer who were treated with pembrolizumab according to standard of care.

METHODS

Patients and trial design

Patients with advanced cancer (solid tumors) and treated with pembrolizumab were included prospectively in the MULTOMAB study (Dutch Trial Registry Number NL6828), who were treated with pembrolizumab between March 2016 and December 2018 at the tertiary referral hospital Erasmus University Medical Center (Rotterdam, The Netherlands) and Amphia Hospital (Breda, The Netherlands). Patients were treated according to standard of care or in a patient early-access program. Combination regimens (e.g. pembrolizumab and platinum-based chemotherapy) were excluded. All patients signed written informed consent provided before study entry. Pembrolizumab was administered as a 3-weekly 30 min infusion of 2 mg/kg or 200 mg fixed dose. Blood sampling was performed prior to every pembrolizumab administration to measure trough drug concentrations. For all patients, blood samples were collected until discontinuation of treatment. Pembrolizumab concentrations were measured in serum by an in-house developed and validated ELISA, with a mean accuracy of 84%–100% from 1 µg/mL up to at least 100 µg/mL, a lower and upper limit of quantification of 0.80 and 100 µg/mL, respectively, and a coefficient of variation of 5.5%.²⁵ This method was additionally validated to assess the effect of freeze thaw cycles prior to pembrolizumab. A lack of effect towards the levels of pembrolizumab up to three freeze thaw cycles was observed (data not shown).

Data collection

At baseline, the following patients' characteristics were collected: gender, age, tumor type, WHO performance status,²⁶ body weight, body surface area (BSA), renal function and laboratory measurements. Laboratory measurements included: serum creatinine, total serum protein,

serum albumin, lactate dehydrogenase (LDH), and leucocyte count. BSA was calculated using the Mosteller formula,²⁷ and renal function was estimated using the Chronic Kidney Disease Epidemiology Collaboration formula.²⁸ OS was calculated to the time of death due to any cause. Response evaluation was performed according to Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST V.1.1).²⁹ PFS was determined by calculating the time from the first administration of pembrolizumab to the time of progressive disease according to RECIST V.1.1 or death due to any cause. BOR was assessed by RECIST V.1.1: a minimum duration of 90 days was required for stable disease and confirmation was not required for partial response or complete response (CR). Data cut-off was per January 1 2020. Immune-related adverse events (irAEs) were reported for grade ≥3 according to the Common Terminology Criteria for Adverse Events V.5.0.

RESULTS

A total of 122 advanced-stage cancer patients, who were treated with either weight-based (2 mg/kg Q3W) or flat dose pembrolizumab (200 mg Q3W), were included in this study. Tumor types included non-small-cell lung cancer (NSCLC) (34%; first or second line depending on the PD-L1 tumor proportion score), melanoma (42%), malignant pleural mesothelioma (MPM; 11%), UCC (12%) and (SCLC; 1%). Weight-based dose scheme was used for treatment of patients with melanoma, whereas the other cancer patients were predominantly treated with fixed dose pembrolizumab. For patients who received at least one prior systemic treatment line (49% of all patients): generally, NSCLC, MPM and UCC patients were pretreated with platinum-based chemotherapy and melanoma patients were pretreated with *BRAF*-inhibitors and/*MEK*-inhibitors. In this real-world data analysis, patients with cerebral metastases at baseline (13.9%), a WHO performance status of >1 (9%) and heavily pretreated patients, were also included. The baseline patient characteristics are shown of all patients in [table 1](#), and also for separate tumor types (NSCLC, melanoma, MPM and UCC; online supplemental table S1).

The median OS of all patients was 2.2 years, which expectedly differed among tumor types online supplemental table S2. Patients had been treated with a median of 9 cycles (range: 2–45) of pembrolizumab. Radiological evaluation was performed to determine the PFS and BOR for this analysis, which was performed for patients with NSCLC or melanoma because of the higher sample size of those groups. The ORR after pembrolizumab treatment in evaluable patients was 40.5% and 49.0% for NSCLC and melanoma, respectively, with a higher proportion of patients with CR (NSCLC n=2; melanoma n=8). Serial serum sampling was performed for all patients to obtain the trough levels of pembrolizumab. A total of 588 observations was available for analysis (with an average of 5 observations per patient; range: 1–11).

Table 1 Baseline characteristics

Categorical covariates		n (%)
Gender		
Male		80 (65.6)
Female		42 (34.4)
Race		
Caucasian		111 (91.0)
Other		3 (1.6)
Unknown		8 (6.6)
Tumor type		
NSCLC		42 (34.4)
Melanoma		51 (41.8)
UCC		15 (12.3)
MPM		13 (10.7)
SCLC		1 (0.8)
Treatment		
Pembrolizumab (2 mg/kg Q3W)		53 (43.4)
Pembrolizumab (200 mg flat Q3W)		69 (56.6)
Prior systemic treatment		
None		62 (50.8)
1		48 (39.3)
2		12 (9.8)
WHO performance status		
0		42 (34.4)
1		54 (44.3)
2		11 (9)
Unknown		15 (12.3)
Cerebral metastases		
Present at baseline		17 (13.9)
Absent at baseline (radiological evaluation was performed)		31 (25.4)
Unknown		74 (60.7)
Continuous covariates	Median (IQR)	n (%)
Age (yr)	69 (57–74)	122 (100)
Body weight (kg)	80 (68–90)	121 (99)
Body surface area (m ²)	1.97 (1.81–2.12)	119 (98)
Creatinine (μmol/L)	82 (69.5–99)	121 (99)
CKD (mL/min)	78 (62.5–92)	121 (99)
Total protein (g/L)	75 (71–79)	99 (81)
Albumine (g/L)	42 (40–45)	114 (93)
LDH (U/L)	238 (190.5–316.5)	120 (98)
Leucocytes (x10 ⁹ cells/L)	8.6 (6.6–10.9)	116 (95)

Baseline covariates of patients. Advanced-stage cancer patients were included that received pembrolizumab monotherapy.

CKD, CKD-EPI renal clearance; LDH, lactate dehydrogenase; MPM, malignant pleural mesothelioma; SCLC, small-cell lung cancer; UCC, urothelial cell cancer.

Of the 122 patients, 600 samples were available. A total of 12 samples were excluded from PK analysis due to missing data (n=5; 0.8%) or sampling artifacts (n=7; 1.2%). A one-compartment model best described the data

(see online supplemental methods). A two-compartment model was unstable as the intercompartmental CL (Q) or peripheral volume of distribution could not be adequately estimated and resulted in poor precision. Both proportional and constant residual error models were evaluated, of which the proportional model performed best. Gender, weight, BSA and albumin on CL and primary tumor (MPM) and LDH on distribution volume (V_d) showed a significant improvement on the structural model with a p<0.001, primary tumors UCC and SCLC on CL showed a significant improvement with a p<0.05 online supplemental table S3. After forward inclusion and backwards elimination, BSA, albumin and UCC on CL and primary tumor (MPM) and LDH on V_d remained in the final model (table 2). The bootstrap estimates are in line with the model parameter estimates (table 2). The GoF-plots of our final model show that the individual predictions are in line with our observations; the residual variability is symmetrically distributed. Some higher variability is observed in the beginning of the treatment prior to steady state (figure 1). The VPC shows the model predictions to be in line with the observations online supplemental figure S1. The final PK model (NONMEM) is available in online supplemental appendix 1.

Additionally, as LDH levels may represent patients with higher tumor burden and thereby altering the target-mediated elimination, an analysis was performed to relate baseline LDH levels to tumor burden and PD-1 positivity in peripheral T cells in a subset of patients with advanced-stage NSCLC. LDH level correlates positively with tumor burden (r 0.6, p=0.001) but negatively with PD-1 positivity in peripheral CD8⁺ lymphocytes (r -0.56, p=0.005; online supplemental table S4).

To determine the relationship of systemic pembrolizumab CL with clinical outcome, individual drug CL obtained from the PK model was used to relate to OS and PFS. In the univariate analysis, patients with higher CL had significantly worse OS than patients with lower CL (HR 1.61; 95% CI 1.26 to 2.05; p<0.001), which remained significant (HR 1.58; 95% CI 1.20 to 2.08; p=0.001) after correction for tumor type and known prognostic factors. Similarly, a significant inverse relationship between CL and PFS was observed in the univariate (HR 1.51; 95% CI 1.15 to 1.98; p=0.003) and multivariate analysis (HR 1.56; 95% CI 1.17 to 2.07; p=0.002). Serum LDH levels remained significant in the multivariate analysis for both OS (analyzed as continuous variable; HR 1.002; 95% CI 1.001 to 1.003; p=0.002) and PFS (HR 1.002; 95% CI 1.001 to 1.003; p<0.001; table 3).

Next, the relationship between systemic pembrolizumab CL and OS was compared between distinct tumor types (table 4). In the multivariate analysis, known prognostic factors were included that may impact the OS of patients with NSCLC, melanoma, MPM or UCC. Patients with NSCLC or MPM with higher CL had significantly worse OS than lower CL (NSCLC: HR 1.69; 95% CI 1.07 to 2.68; p=0.024, MPM: HR 3.29; 95% CI 1.08 to 10.09; p=0.037) after correction for patient factors, while this effect could

Table 2 Population PK parameters for pembrolizumab							
Parameters	Nonmem			Bootstrap			
	Estimate	RSE (%)	95% CI (lower)	95% CI (upper)	Shrink. (%)	Median	95% CI (lower) 95% CI (upper)
Pop parameters							
CL (L/day)	0.257	3.3	0.24	0.26	–	0.256	0.24 0.27
V (L)	6.8	8.6	5.66	7.95	–	6.9	5.31 8.71
Covariates							
BSA~CL	1.46	24	0.78	2.14	–	1.50	0.80 2.20
Albumin~CL	–1.43	27	–2.19	–0.67	–	–1.40	–2.31 –0.75
UCC~CL	1.29	11	1.02	1.56	–	1.28	1.02 1.62
Meso~V	0.58	23	0.32	0.84	–	0.57	0.32 0.9
LDH~V	0.34	21	0.20	0.48	–	0.33	0.14 0.52
Variability							
IIV CL (%)	31	8.5	25	36	8	30	24 35
IIV V (%)	29	11	22	35	30	28	21 36
Residual error	0.17	7	0.14	0.19	14	0.16	0.14 0.19

Showing the population parameters estimates, covariate effects and inter-individual variability of the final population PK model. BSA, body surface area; CL, clearance; CV%, percentage coefficient of variation; IIV, inter-individual variability; LDH, lactate dehydrogenase; Meso, malignant pleural mesothelioma; PK, pharmacokinetic; RSE, relative SE; UCC, urothelial cell carcinoma; V, volume of distribution.

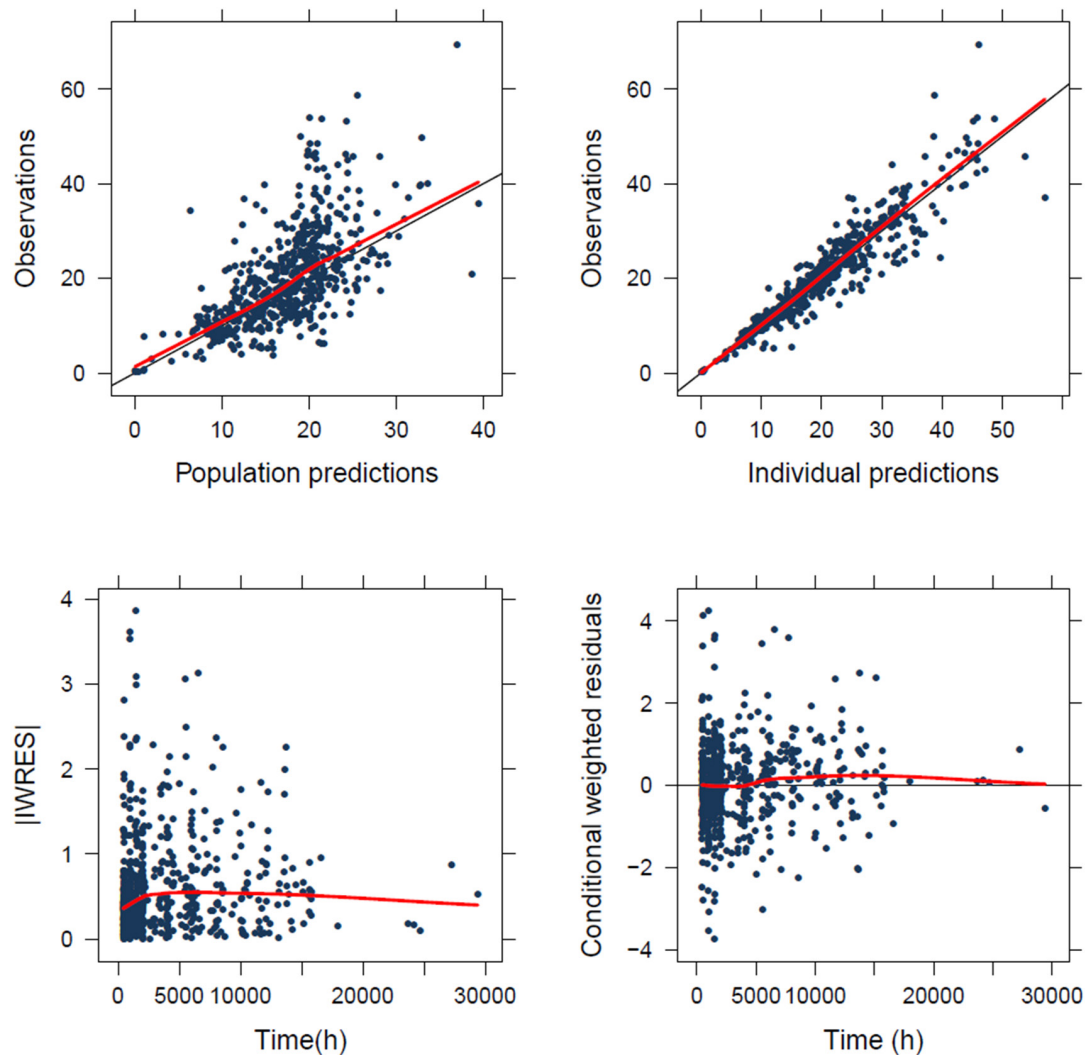


Figure 1 Standard goodness of fit of the PK model for pembrolizumab. Goodness-of-fit plots of the final model. (A) Predicted population concentrations versus observed concentrations of the final model; (B) Predicted individual concentrations vs observed concentrations of the final model. (C) Individual weighted residuals (IWRES) versus time (h=time in hours), (D) conditional weighted residuals versus time (h=time in hours). Residual variability remains (outside of the -2.2 conditional weighted residuals), especially in the beginning of the treatment. PK, pharmacokinetic.

not be observed for melanoma (HR 1.31; 95% CI 0.85 to 2.04; $p=0.22$) after correction for patient factors, and neither for UCC (univariate: HR 1.30; 95% CI 0.75 to 2.25; $p=0.34$). For NSCLC and melanoma, the OS and PFS curves grouped by the quartile of CL (Q1 represents patients with the lowest quartile of CL, whereas Q4 the highest quartile of CL) are shown, as well as the distribution of CL (L/day) across BOR groups (online supplemental figure S2).

Severe irAEs (defined as grade ≥ 3) were observed in 17% ($n=21$) of the evaluated patients during follow-up (see online supplemental table S2) for an overview of the distribution across tumor types). Of those with severe irAEs, most patients had gastrointestinal toxicity (8%), but also immune-related endocrinopathies (3%), hepatotoxicity (3%), pneumonitis (3%), and skin toxicity (3%) were reported. Additionally, three patients had grade ≥ 3 neurological, renal or rheumatological toxicity (online

supplemental table S5). No difference was observed in drug CL of patients with severe irAEs compared with patients without or low-grade irAEs (mean CL 0.29 vs 0.30 L/day, $p=0.70$).

DISCUSSION

The present study describes the first real-world PK model of pembrolizumab. Unlike previously published PK analyses that were based on trial data with strict inclusion criteria, this study also included patients with worse performance at baseline and patients with various tumor types. When building our model, we observed that BSA and serum albumin levels had a significant effect on systemic CL of pembrolizumab, whereas serum LDH levels had a significant increasing effect on the volume of distribution. When using our final model, incorporating the above parameters, we

Table 3 Univariate and multivariate OS and PFS analysis

		Univariate analysis			Multivariate analysis		
Parameters	Test variables	HR	95% CI	P value	HR	95% CI	P value
OS							
Drug clearance	Q4 vs. Q3 vs. Q2 vs. Q1	1.61	1.26 to 2.05	<0.001*	1.58	1.20 to 2.08	0.001*
Tumor type	NSCLC vs. other	1.07	0.61 to 1.88	0.80	0.79	0.29 to 2.14	0.64
	Melanoma vs. other	0.48	0.28 to 0.85	0.01*	0.53	0.20 to 1.40	0.19
	MPM vs. other	2.24	1.09 to 4.61	0.03*	2.59	0.83 to 8.09	0.10
	UCC vs. other†	1.95	0.98 to 3.88	0.06			
Prior treatment	2 vs. 1 vs. none	1.85	1.27 to 2.69	0.001*	1.24	0.75 to 2.06	0.39
Age (years)	Continuous	0.998	0.98 to 1.02	0.87			
Gender	Male vs. female	1.05	0.60 to 1.82	0.87			
WHO	2 vs. 1 vs. 0	1.43	0.94 to 2.16	0.09	0.99	0.57 to 1.73	0.98
Smoking	Never vs. current/former	0.72	0.29 to 1.83	0.49			
LDH (U/L)	Continuous	1.001	1.000 to 1.002	0.005*	1.002	1.001 to 1.003	0.002*
PFS							
Drug clearance	Q4 vs. Q3 vs. Q2 vs. Q1	1.51	1.15 to 1.98	0.003*	1.56	1.17 to 2.07	0.002*
Tumor type	NSCLC vs. melanoma	1.28	0.72 to 2.29	0.39			
Prior treatment	2 vs. 1 vs. none	1.59	1.02 to 2.46	0.04*	1.29	0.81 to 2.08	0.29
Age (years)	Continuous	0.99	0.97 to 1.02	0.50			
Gender	Male vs. female	1.07	0.59 to 1.96	0.82			
WHO	2 vs. 1 vs. 0	1.08	0.70 to 1.67	0.73			
Smoking	Never vs. current/former	1.65	0.77 to 3.54	0.20			
LDH (U/L)	Continuous	1.002	1.000 to 1.002	<0.001*	1.002	1.001 to 1.003	<0.001*

Table showing the results of the univariate and multivariate Cox regression survival analysis for OS and PFS. Tumor type (NSCLC, melanoma, MPM and UCC) integrated as a test variable and contrasted to all other patients in the dataset, to allow for correction of tumor type in the multivariate analysis. Parameters (including individual drug clearance and known prognostic factors) with a $p < 0.10$ are incorporated in the multivariate analysis. The HR is shown, with the 95% CI and the p value (p).

*Significance ($p < 0.05$).

†Not computed in the multivariate analysis, linearly dependent on other test variables for the parameter.

LDH, lactate dehydrogenase; LDH, lactate dehydrogenase; MPM, malignant pleural mesothelioma; NSCLC, non-small cell lung cancer; OS, overall survival; PFS, progression-free survival; UCC, urothelial cell carcinoma.

determined a profound covariate effect towards CL and V_d of respectively UCC and MPM, when compared with other tumor types. Finally, a strong negative relationship was determined between systemic CL and clinical outcome after pembrolizumab treatment, which was more profound in patients with NSCLC or MPM than melanoma or UCC.

Our one-compartment PK model was developed with a similar performance and comparable covariate effects as found in previous studies with PD-1 inhibitors. An overview of the population PK parameters tested previously, as well as the covariates that were tested and validated in our study, are listed in table 5. We demonstrated that BSA and serum albumin levels at baseline showed a positive correlation (covariate estimate BSA: 1.46) and a negative correlation (albumin: -1.43) with systemic CL of pembrolizumab, respectively, which was consistent with earlier models,^{19 21–24} implicating that patients with a higher BSA or lower serum albumin levels before treatment will have a

higher rate of pembrolizumab elimination, and vice versa. Serum LDH levels at baseline had a significant covariate effect (0.34, 95% CI 0.20 to 0.48) on the V_d of pembrolizumab.

Serum LDH levels are considered as a negative prognostic factor for metastatic melanoma^{30–33} and correlates positively with tumor burden.³⁴ No differences in ICI efficacy for metastatic melanoma have been observed in a subgroup analysis between patients with normal and elevated LDH,³⁵ which supports a prognostic (and not predictive) value for LDH in metastatic melanoma. While LDH is in lesser extent prognostic in patients with other tumor types, our data demonstrates the effect of LDH on the PK of pembrolizumab across solid tumors, including NSCLC. While LDH has been suggested to serve as a predictive biomarker to select NSCLC patients who benefit from ICIs based on a meta-analysis,³⁶ accumulating data suggest only prognostic value of LDH.³⁷ Although previous PK models did not test the covariate effect of LDH, baseline tumor burden (which was

Table 4 Univariate and multivariate OS analysis for separate solid tumors

		Univariate analysis			Multivariate analysis		
Parameters	Test variables	HR	95% CI	P value	HR	95% CI	P value
NSCLC (n=42)							
Drug clearance	Q4 vs. Q3 vs. Q2 vs. Q1	1.68	1.07 to 2.64	0.025*	1.69	1.07 to 2.68	0.024*
Age (years)	Continuous	1.06	1.002 to 1.13	0.04*	1.07	1.002 to 1.13	0.04*
Gender	Male vs. female	1.19	0.46 to 3.08	0.72			
WHO	2 vs. 1 vs. 0	0.52	0.22 to 1.20	0.13			
Smoking	Never vs. current/former	5.69	0.69 to 47	0.11			
Melanoma (n=51)							
Drug clearance	Q4 vs. Q3 vs. Q2 vs. Q1	1.49	0.99 to 2.25	0.056	1.31	0.85 to 2.04	0.22
Age (years)	Continuous	0.98	0.95 to 1.02	0.29			
Gender	Male vs. female	0.89	0.36 to 2.17	0.79			
WHO	2 vs. 1 vs. 0	3.36	1.40 to 8.05	0.007*	2.21	0.87 to 5.60	0.10
LDH (U/L)	Continuous	1.003	1.001 to 1.004	0.002*	1.003	1.000 to 1.005	0.026*
Malignant pleural mesothelioma (n=13)							
Drug clearance	Q4 vs. Q3 vs. Q2 vs. Q1	3.58	1.29 to 9.97	0.015*	3.29	1.08 to 10.09	0.037*
Age (years)	Continuous	0.90	0.80 to 0.998	0.04*	0.973	0.87 to 1.09	0.64
Gender	Male vs. female	1.32	0.16 to 10.78	0.79			
WHO	2 vs. 1 vs. 0	2.07	0.50 to 8.64	0.32			
UCC (n=15)							
Drug clearance	Q4 vs. Q3 vs. Q2 vs. Q1	1.30	0.75 to 2.25	0.342			
Age (years)	Continuous	1.01	0.95 to 1.07	0.73			
Gender	Male vs. female	0.06	0.01 to 0.59	0.016*	0.06	0.01 to 0.59	0.016*
WHO	2 vs. 1 vs. 0	1.56	0.32 to 7.59	0.58			

Table showing the results of the univariate and multivariate Cox regression survival analysis of drug clearance for OS, stratified by tumor type. For each tumor type, parameters (including individual drug clearance and known prognostic factors) with a $p < 0.10$ are incorporated in the multivariate analysis. The HR is shown, with the 95% CI and the p value.

*Significance ($p < 0.05$).

LDH, lactate dehydrogenase; NSCLC, non-small cell lung cancer; PFS, progression-free survival; UCC, urothelial cell carcinoma.

determined by the sum of longest diameters of a small selection of target lesions) was reported to have a positive covariate effect on CL.^{21 24} Taken together, this may suggest that patients with higher LDH levels have higher tumor burden, with altered target-mediated elimination by binding of pembrolizumab to its target receptor PD-1. To address this, the correlation of baseline LDH levels was additionally assessed with tumor burden and peripheral PD-1 expression. Indeed, a positive and significant correlation was found for LDH levels and tumor burden, whereas an inverse and significant correlation for LDH levels and PD-1 receptor positivity in peripheral CD8⁺ lymphocytes. Another explanation may be that patients with higher LDH levels demonstrate a more advanced disease, with a higher likelihood of cancer cachexia resulting in higher drug CL. Cancer cachexia is characterized by catabolism of endogenous immunoglobulins through its metabolic breakdown into amino acids.³⁸ Similar mechanisms have been suggested for pembrolizumab, being an exogenous IgG₄ immunoglobulin.²⁰ Compellingly, our findings do not only implicate the

covariate effect of LDH on the V_d , but also demonstrate a negative correlation of serum albumin with systemic CL of pembrolizumab. The same elimination and recycling mechanisms of endogenous immunoglobulins via the FcRn receptor are considered to play a key role in the metabolism of albumin in blood.^{39 40} Further pharmacodynamic research is needed to confirm and evaluate the covariate effect of LDH on the volume of distribution and the covariate effect of albumin on drug CL.

A positive effect of the renal function on CL, which was previously reported but discussed to have no clinically relevant effect,^{21 24} could not be confirmed in the present PK analysis. Renal excretion of monoclonal antibodies is not expected because of the large molecular size.⁴¹

The present study is consistent with previous research suggesting that the inverse relationship between CL and clinical outcome is affected by the metabolic state of patients, which may change clinical practice of ICIs, for instance by dose escalation or the application of a loading dose. However, the beneficial of such an intervention seems low since a dose-response relationship

Table 5 Literature overview of previous PK model parameters for PD-1 inhibitors

Data source	N	Obs.(obs./pt.)	Dose range	PK model	CL (L/day)	Vc (L)	Vp (L)	Q (L/day)	Variance of CL (CV%)	Covariates tested	Covariates in model	Reference
Pembrolizumab:												
Trial*	1223	10.0	1–10 mg/kg Q3W	2-comp, LE	0.22	3.48	4.06	0.795	38	Age, gender, ethnicity, renal function, liver tests, albumin, glucocorticoid use, prior treatment, tumor type, PS, TB	Albumin (neg on CL), PS (neg on CL), renal function (pos on CL), prior ipilimumab (pos on CL), gender (female: neg on CL), TB (pos on CL)	Ahamadi et al ²¹
Trial†	Not given	–	0.02–10 mg/kg Q3W	2-comp, LE+NLE	0.168	2.88	2.85	0.384	28.8	–	–	Elassais-Schaap et al ²³
Trial†	2841	6.1	1–10 mg/kg Q3W	2-comp, LE, TV	0.249	3.47	2.96	0.889	30.7	Age, gender, ethnicity, body weight, renal function, liver tests, glucocorticoid use, PS, geographics, smoking, PD-L1, lymphocyte count (TV), LDH (TV), albumin (TV), TB (TV)	Albumin (neg on CL), bilirubin (neg on CL), renal function (pos on CL), PS (pos on CL), gender (female: neg on CL), TB (pos on CL)	Li et al ²⁴
Nivolumab:												
Real-world	221	7.8	3 mg/kg Q2W	2-comp, LE	0.211	3.46	3.46	0.48	30.7	Age, gender, ethnicity, body weight, BSA, renal function, total serum protein, albumin, LDH, tumor type, PS, TB, leukocyte count	Gender (female: neg on CL), BSA (pos on CL), albumin (neg on CL)	Hurkmans et al ¹⁹
Trial‡	1895		0.1–20 mg/kg Q2W	2-comp, LE	0.23	3.63	2.78	0.770	35	Age, gender, ethnicity, body weight, renal function, liver tests, PS, TB, LDH, tumor type, PD-L1	Body weight (pos on CL), renal function (pos on CL), performance (pos on CL), ethnicity (asian: neg on CL), gender (female: neg on CL)	Bajaj et al ²²

Population PK parameters of previous models for PD-1 inhibitors (i.e. pembrolizumab and nivolumab).
*KEYNOTE-001–002, and –006.
†KEYNOTE-010.
‡Pooled data from eleven phase I–III.
BSA, body surface area; CL, systemic clearance; LDH, lactate dehydrogenase; LE, linear elimination; NLE, non-linear elimination; obs, observations; PD-L1, programmed cell death protein 1; PS, performance status; Q3W or Q2W, 3 or 2 weekly; TB, tumor burden; TV, time variance; Vc or Vp, central or peripheral volume of distribution.

has not been identified for pembrolizumab. While serum albumin may be well used as a surrogate for the metabolic state of patients, further research is needed to integrate robust classification of cancer cachexia in PK models.

As expected, no association could be determined between the PK of pembrolizumab and the occurrence of severe irAEs. ICI are relatively well-tolerated with a high maximum tolerated dose. Only one⁴² of the 13 main dose-finding studies of ICI monotherapy identified DLTs.³ In the current study, 21 patients (17%) of all patients had grade 3 or 4 irAEs, and these occurred generally after 4.5 months (median) ranging from 2 weeks up to 2 years after start of treatment, which was comparable with previous observations in this setting.⁴³

All studies have limitations, including this one. Importantly, correlating systemic CL with clinical outcome using a PK model might result in a confounded relationship with baseline prognostic factors and/or cancer disease severity. We demonstrated a strong relationship between drug CL and clinical outcome, which was most profoundly present in NSCLC and MPM patients. Although subtle difference between dose levels and objective response across tumor types are demonstrated in numerous dose finding phase I/II trials, such analyses in small patients cohorts should be interpreted with care, including subgroup analyses presented in this study. Furthermore, no comparisons could be made between fixed- and weight-based dose schemes. The dosing scheme had been selected based on the local protocol, which differed between departments based on approved indications, and thus significant differences of dose schemes were only regarded between tumor types. However, in this study, BSA was found to be a better body size measure compared with weight in explaining interpatient variability. In that respect, linear weight based dose individualization seems not rational. Long-term sampling during the entire treatment period was performed on a total of 122 patients, this may be considered as a small study cohort compared with former models based on trial data. Moreover, we developed a one-compartment model—rather than a two-compartment model—because trough levels had been determined over time, which did not permit us to estimate the central and peripheral volume of distribution. Yet, our one-compartment model worked with sufficient accuracy and allowed us to perform the study as a part of routine clinical care in unselected patients.

The current report independently investigated the effect of patient factors on the PK of pembrolizumab, suggesting the feasibility and importance of studying the PK by serial blood sampling in a real-world setting. This is particularly important for novel ICIs (including current combinations with chemotherapy) where the study population not always reflects a representative sample of the target population. In this study we found that part of the interindividual variability in pembrolizumab PK can be explained by BSA, serum albumin

and LDH. We demonstrated a strong inverse CL-OS relationship for predominantly NSCLC and MPM.

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Contributors DPH wrote the manuscript. DPH, RHJM, JGJVA, MJ and SLWK contributed to the conception and design of the study. DPH, SDTS and SLWK contributed to the acquisition, analysis, or interpretation of data. DPH, RHJM, JGJVA, CHVdL, KdJ, AAMVdV and RD contributed in interpretation of the data. DPH, KdJ, LP, EAB, AAMVdV and RHJM performed radiological evaluation. MWJS, AJMW contributed technical and material support and performed the measurements of pembrolizumab concentrations. All authors contributed to revision of the manuscript. All authors read and approved the final manuscript.

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SUPPLEMENTARY METHODS

Population pharmacokinetic modelling

Population PK analysis was performed with nonlinear effects modelling approach in NONMEM® version 7.3 (ICON, Development Solutions, MD, USA). Pirana software version 2.9.5b (Certara, NJ, USA) was used as a modeling environment, and data were further handled in R studio version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria). The first-order conditional estimates method with interaction (FOCE+I) was used for parameter estimation(1, 2). Initially a one-compartment model was fitted to the pembrolizumab concentration-time data. Subsequently, more complex models were fitted to the data. Improvements of the fit and evaluation of the models was done quantitatively through parameter precision, change in objective function value (OFV), shrinkage, conditional number, and changes in interindividual and residual variability. The Likelihood ratio test was used, which assumes the difference in OFV between hierarchical models Chi square distributed. A decrease of 3.84-points in OFV for one degree of freedom with a p-value of < 0.05 was considered a significant improvement of fit. Model improvement was evaluated visually through goodness-of-fit plots (GoF), quantile-quantile (QQ) plots and visual predictive checks (VPC). Both constant and proportional error models were evaluated for interindividual variability (IIV). A proportional error model was used for the residual variability.

After finalization of the structural model, the covariate model was built. The evaluation of covariates was done through iterative forward inclusion (p-value < 0.05) and backwards elimination (p-value < 0.01). Missing covariate values were replaced by the median covariate value of the population. Missing categorical covariates were excluded, however none were present. The continuous covariates BSA, albumin, creatinine, weight, renal function and LDH were tested on CL and volume of distribution (V_d). Continuous covariates were implemented as shown in **equation 1**. Similarly, the categorical covariates gender, WHO performance status and primary tumor type were tested on CL and V_d . Categorical covariates were implemented as shown in **equation 2**. The robustness of the parameter estimates was evaluated using a non-parametric bootstrap procedure (n=1,000). A VPC was performed for internal validation of the model.

Eq.1

$$P_i = \theta_{pop} * \left(\frac{COV}{median\ COV} \right)^{\theta_{cov}} * exp(\eta_i)$$

Eq.2

$$P_i = \theta_{pop} * \theta_{cat}^{FLAG} * exp(\eta_i)$$

P_i is the individual parameter estimate, θ_{pop} is the population estimate. COV is the continuous covariate, θ_{cov} is the estimated exponent parameter of the continuous covariate. θ_{cat} is the estimated parameter of the categorical covariate. FLAG is used to switch the categorical covariate estimate ON (FLAG=1) or OFF (FLAG=0). η_i is the individual deviation from the population estimate.

Statistical analysis

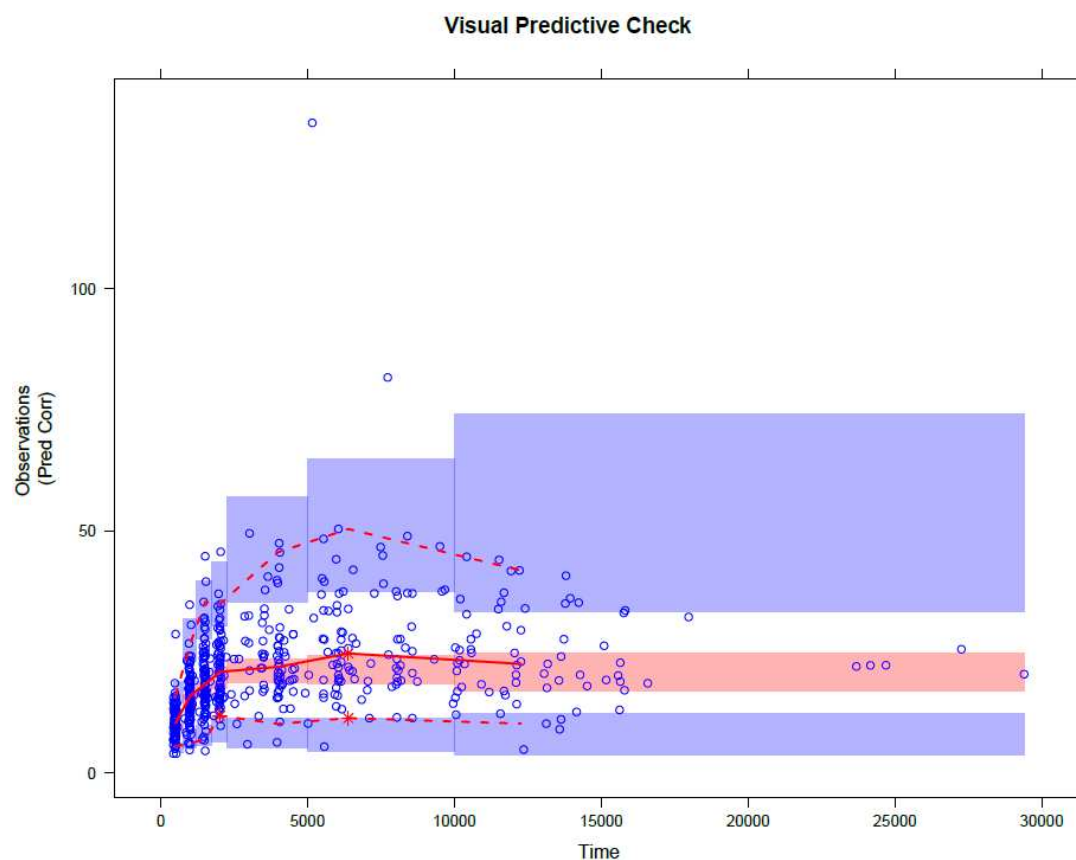
Descriptive statistics comprised of frequency, median, range or inter-quartile range (IQR) of covariates. Descriptive statistics were shown for all patients and after stratification by tumor type. All patients were included in the PK model. Univariate and multivariate Cox regression was performed to investigate the relationship between systemic drug clearance (quartiles of CL based on the final PK model) and clinical outcome (PFS and OS), correction for known prognostic factors was applied that meet a p-value of < 0.10. Importantly, differences in the clinical outcome of different cancer type were considered by including tumor type as a test parameter (NSCLC, melanoma, malignant pleural mesothelioma (MPM) or urothelial cell carcinoma [UCC]). Separate OS analyses were performed on NSCLC, melanoma, MPM and UCC. The survival curves and distribution of CL (L/day) over BOR groups was visualized in separate tumor types with a sufficient number of patients (being NSCLC and melanoma). Lastly, the relationship between systemic pembrolizumab clearance and irAEs was assessed using independent samples t-tests. Generally, a two-sided p-value < 0.05 was considered significant.

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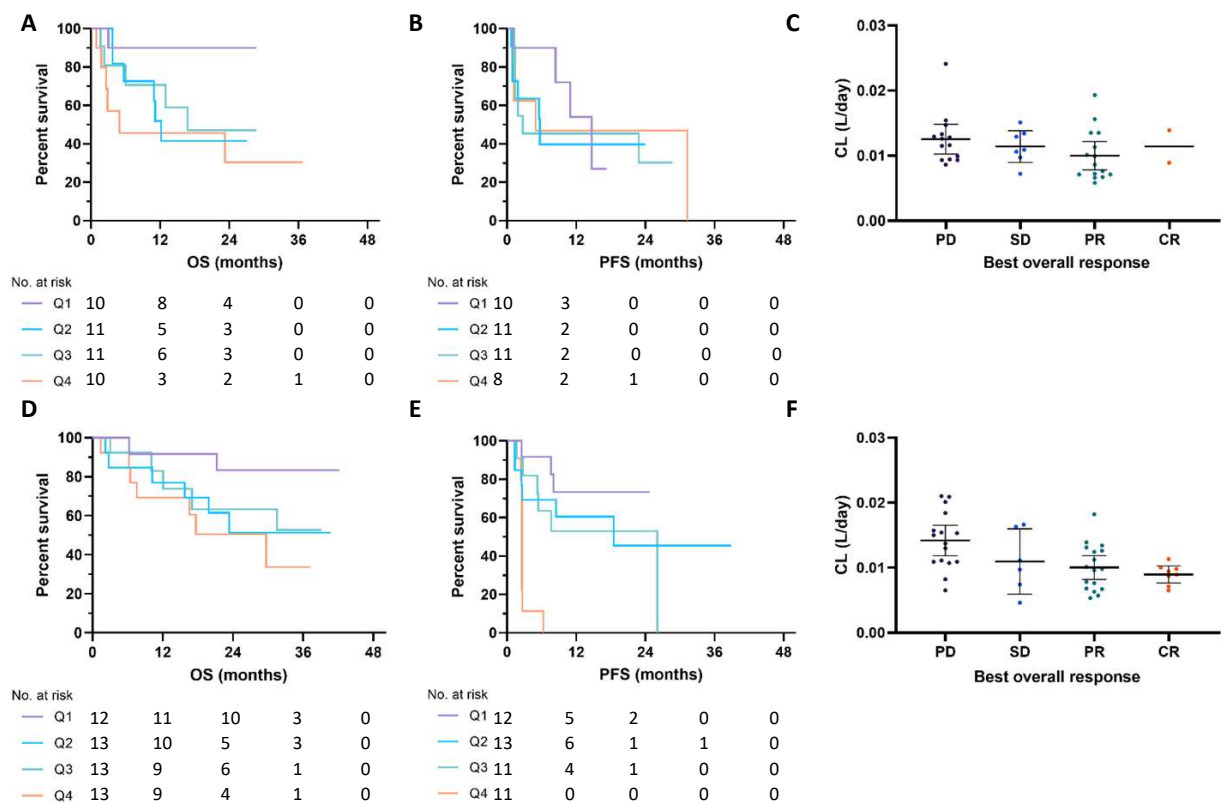
SUPPLEMENTARY DATA

Supplementary Fig. S1. Visual predictive check of the final PK model



Supplementary Fig. S1: Prediction corrected visual predictive checks, showing the fit of predicted serum concentrations of pembrolizumab ($\mu\text{g/mL}$) versus the observed concentrations of the final model over time. The predictions are in line with the observed data. However, the lower boundary of the confidence interval of the model prediction is rather low with respect to the observations. This is also the case for median, especially between 5000-10000 h. The red solid line indicates the median observed concentrations and the surrounding opaque red area the simulation based 95% interval for the median. The red dashed lines indicate the observed 2.5% and 97.5% percentiles and the surrounding opaque blue areas show the simulated 95% confidence intervals for the corresponding predicted percentiles. One extreme predicted corrected observation value was excluded from graph.

Supplementary Fig. S2. Relationship between pembrolizumab CL and clinical outcome



Supplementary Fig. S2: Graphs showing the relationship between drug clearance and clinical outcome measures for NSCLC (upper part) and melanoma (lower part). Kaplan-Meier curves are stratified by quartiles of clearance: Q1 represents the first quartile (lowest clearance) – Q4 represents the fourth quartile (highest clearance). A) overall survival (OS; in months), B) progression-free survival (PFS; months) and C) best overall response (BOR) of patients with non-small cell lung cancer (NSCLC); D) OS, E) PFS and F) BOR of patients with melanoma. Bars indicate the 95% confidence interval of the mean. Abbreviations: progressive disease (PD), stable disease (SD), partial response (PR) and complete response (CR).

Supplementary Table S1. Baseline characteristics by tumor type

Categorical characteristics	NSCLC	Melanoma	MPM	UCC
	n	n	n	n
Gender				
Male	25	32	11	12
Female	17	19	2	3
Treatment				
Pembrolizumab (dose 2 mg/kg Q3W)	2	51	-	-
Pembrolizumab (dose 200 mg Q3W)	40	-	13	15
Prior systemic treatment				
None	27	34	0	1
1	14	15	10	11
2	1	2	3	3
WHO Performance Status				
0	6	30	1	5
1	24	15	5	10
2	9	1	1	-
Unknown	3	5	6	-
Continuous characteristics	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)
Age (yr)	69.5 (61-72)	67 (55-77)	70 (61.5-74.5)	68 (55-74)
Body Surface Area (m²)	1.9 (1.7-2.0)	2.0 (1.8-2.2)	2.0 (1.9-2.2)	2.1 (1.8-2.3)
Albumine (g/L)	40 (36-44)	43 (41-46)	42 (40.3-43)	43 (41-45)
LDH (U/L)	258 (212-350)	256 (198-331)	203 (157-225)	200 (165-244)

Supplementary Table S1: Table showing the baseline patient characteristics, stratified by tumor type.

Abbreviations: lactate dehydrogenase (LDH), non-small cell lung cancer (NSCLC), urothelial cell carcinoma (UCC), number (n), interquartile range (IQR), every three weeks (Q3W).

Supplementary Table S2. Distribution of clinical outcome (BOR, PFS and OS) and irAEs

	NSCLC median (SE)	Melanoma median (SE)	MPM median (SE)	UCC median (SE)
OS (months)	22.7 (2.5) ^a	29.9 (2.2) ^a	7.2 (1.2)	16.4 (6.3)
PFS (months)	11.0 (5.7)	8.5 (6.6)	-	-
	n (%)	n (%)	n (%)	n (%)
BOR				
PD	14 (33.3)	16 (31.4)	-	-
SD	7 (16.7)	6 (11.8)	-	-
PR	15 (35.7)	17 (33.3)	-	-
CR	2 (4.8)	8 (15.7)	-	-
NE	4 (9.5)	4 (7.8)	-	-
irAEs				
Grade ≥3	9 (21.4)	7 (13.4)	2 (15.4)	3 (20)
Grade <3	28 (66.7)	44 (86.3)	11 (84.6)	12 (80)
Unknown	5 (11.9)	-	-	-

Supplementary Table S2: The table shows the clinical outcome measures for separate patient groups after treatment of pembrolizumab, stratified by tumor type. Best overall response (BOR) evaluation was performed by RECIST v1.1. Abbreviations: non-small cell lung cancer (NSCLC), malignant pleural mesothelioma (MPM), urothelial cell carcinoma (UCC), standard error (SE), number (n), progression-free survival (PFS), overall survival (OS), progressive disease (PD), stable disease (SD), partial response (PR), complete response (CR), non-evaluable by RECIST v1.1 (NE). ^aThe mean (SD of the mean) was shown, as the median OS was not reached for this group.

Supplementary Table S3. Parameter estimates

	Covariate effect on CL		Covariate effect on V	
	Estimates	dOFV	Estimates	dOFV
Categorical variables				
Female vs. male	0.72	-16.4***	1.02	-0.03
Melanoma	0.89	-2.22	1.08	-0.78
MPM	1.26	-3.31	0.51	-20.9***
UCC	1.28	-4.86*	1.02	-0.01
NSCLC	0.94	-0.56	1.17	-2.90
SCLC	0.42	-4.12*	1.02	-0.002
Continuous variables				
Age (yr)	-0.11	-0.26	-0.28	-1.75
Body Weight (kg)	0.70	-15.74***	0.28	-1.61
Body Surface Area (m ²)	1.38	-19.43***	0.33	-0.68
Creatinine (μmol/L)	0.188	-2.72	0.20	-2.04
CKD (mL/min)	-0.04	-0.11	0.13	-0.62
Total Protein (g/L)	0.46	-0.76	-0.74	0.75
Albumine (g/L)	-1.22	-10.45***	-0.72	-1.98
LDH (U/L)	0.002	-0.001	0.39	-20.9***

Supplementary Table S3: Effect and estimate of the individual categorical and continuous covariates

*when implemented on the CL and V of the structural model. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.*

Abbreviations: change in objective function value (dOFV), non-small cell lung cancer (NSCLC), mesothelioma (MPM), urothelial cell carcinoma (UCC), body surface area (BSA), CKD-EPI renal clearance (CKD), lactate dehydrogenase (LDH).

Supplementary Table S4. Correlation of LDH with volumetric tumor burden and peripheral PD-1 expression on lymphocytes

Parameter		Test variables		
		Volumetric tumor burden	Fraction PD-1 positive CD4 lymphocytes	Fraction PD-1 positive CD8 lymphocytes
LDH levels	Pearson correlation	0.63	0.19	-0.56
	P-value (2-sided)	0.001**	0.38	0.005**
	N	24	24	24

*Supplementary Table S4: Correlation matrix of lactate dehydrogenase (LDH) levels at baseline versus volumetric tumor burden, the fraction PD-1 positive CD4 T cells and the fraction PD-1 positive CD8 T cells (peripheral). The volumetric tumor burden of all primary or metastatic lesions was determined in a separate cohort of 24 stage IV NSCLC patients, as previously reported. Pearson correlation test was performed, significance of <0.01 was indicated by **.*

Supplementary Table S5.

irAEs	Grade 3 n	Grade 4 n	(%)
Gastrointestinal toxicity	9	-	(36)
Immune-related skin toxicity	4	-	(16)
Immune-related endocrinopathies	2	1	(12)
Immune-related hepatotoxicities	3	-	(12)
Immune-related pneumonitis	3	-	(12)
Neurological toxicity	1	-	(4)
Renal toxicity	1	-	(4)
Rheumatological toxicity	1	-	(4)

Supplementary Table S5: A total of 21 patients (17%) had grade 3 or 4 immune-related adverse events (irAEs). As more than one irAE may be reported for an individual patient, a total of 25 irAEs were reported, the majority being gastrointestinal toxicity.

Appendix I. Syntax of PK model for pembrolizumab*Appendix I:*

\$PROBLEM PK model Pembrolizumab

\$INPUT

CENSOR

ID

DROP

TIME

TAD

AMT

RATE

DV

EVID

MDV

AGE

HT

WT

BSA

GNDR

CYCLE

TVS=DROP

CREAT

CKD

TPRO

ALB

LDH

LEU

TMR

\$DATA pembro_db_140420.CSV IGNORE=C

\$SUBROUTINES

ADVAN1 TRANS2 ; 1-compartment iv data

\$PK

; Flags for different tumor types for implementation into covariate analysis

FLAG1 = 0

FLAG2 = 0

FLAG3 = 0

FLAG4 = 0

FLAG5 = 0

FLAG6 = 0

IF(TMR.EQ.1)FLAG1 = 1 ; Melanoma

IF(TMR.EQ.2)FLAG2 = 1 ; Mesothelioma

IF(TMR.EQ.3)FLAG3 = 1 ; NSCLC


```

IF(TMR.EQ.4)FLAG4 = 1 ; SCLC
IF(TMR.EQ.5)FLAG5 = 1 ; UCC
IF(TMR.EQ.6)FLAG6 = 1 ; Unknown

```

; Continuous covariates centered around median. Missing values replaced by median.

```

IF(BSA.EQ.-99) THEN
  BSAX = 1.96
ELSE
  BSAX = BSA
ENDIF

```

```

IF(WT.EQ.-99) THEN
  WTX = 80
ELSE
  WTX = WT
ENDIF

```

```

IF(ALB.EQ.-99) THEN
  ALBX = 43
ELSE
  ALBX = ALB
ENDIF

```

```

IF(LDH.EQ.-99) THEN
  LDHX = 78
ELSE
  LDHX = LDH
ENDIF

```

```

TVCL = THETA(3)* (BSAX/1.96)**THETA(6)* (ALBX/43)**THETA(7)* THETA(9)**FLAG5
CL = TVCL*EXP(ETA(1))

```

```

TVV = THETA(4) * THETA(5)**FLAG2* (LDHX/78)**THETA(8)  V = TVV* EXP(ETA(2))

```

```

S1 = V

```

```

$THETA
(0, 0.15) ;1 prop
(0 FIX) ;2 add
(0, 0.01) ;3 CL
(0, 7) ;4 V
(-1, 1) ;5 Meso
(-1, 1) ;6 BSA
(-10, 1) ;7 ALB
(-1, 1) ;8 LDH
(-1, 1) ;9 UCC

```

```

$OMEGA BLOCK(2)
0.03 ; IIV-CL
0.01 0.03 ; IIV-V

```

```

$SIGMA

```

1 FIX

\$ERROR

IPRED = F

IRES = DV-IPRED

W = IPRED*THETA(1)+THETA(2)

IF (W.EQ.0) W = 1

IWRES = IRES/W

Y= IPRED+W*ERR(1)

\$EST METHOD=1 MAXEVAL=99999 SIG=3 PRINT=5 NOABORT POSTHOC INTERACTION

\$COV PRINT=E UNCONDITIONAL