SUPPLEMENTARY DATA

Assessment of pembrolizumab binding to immunoglobulin superfamily members

Binding of pembrolizumab to PD-1, CD28, CTLA-4, and ICOS was assessed in electrochemiluminescence-based (ECL) immunoassays. Recombinant human PD-1 Fc chimera, recombinant human CTLA-4 Fc chimera, recombinant human CD28 Fc chimera, and recombinant human ICOS Fc chimera (all from R&D Systems) were immobilized onto 96-well Small Spot High Bind plates (Meso Scale Discovery) by adding 25 µL of 1 µg/mL solutions into each well and incubating overnight at 4°C. After washing 3 times with PBS/0.05% Tween 20, plates were blocked in PBS/0.5% bovine serum albumin (BSA) for approximately 1 hour at room temperature. After further washing, plates were prepared for the binding experiment. For each experiment, antibodies were diluted in assay buffer, PBS containing 0.5% BSA (w/v), 0.05% Tween 20 (v/v), 0.25% CHAPS (w/v), and 5 mM EDTA to concentrations of 10000, 2500, 625, 156.3, 39.1, 9.8, and 2.4 ng/mL. Twenty-five microliters of each antibody solution or assay buffer was added into the appropriate wells. Plates were incubated for approximately 1 hour at room temperature and then washed 3 times. After washing, 150 µL of Read Buffer T with Surfactant (Meso Scale Discovery) was added into each well, and plates were processed using the Meso Scale Discovery MESO SECTOR S 600; the results were analyzed using Discovery WORKBENCH® Software 4.0.12.1. A human IgG4 antibody directed to RSV was used as a negative control antibody (MSD). Commercially available antibodies (R&D Systems) directed to CD28, CTLA-4, or ICOS were used as positive control antibodies. Experiments were conducted 3 times in duplicate. Statistical analyses were performed using Prism software (GraphPad).

Supplementary Data Figure S1 shows that pembrolizumab does not bind to immunoglobulin superfamily members human CD28, human CTLA-4, or human ICOS.

In Vitro Activity Assays

*Activity on primary T cell clone* *BC4-49 (T cell clone co-culture assay)*. This section contains additional detail for the methods used for the T cell clone co-culture assay. Human CD4+, HLA-DR alloantigen-specific T cell clone BC4-49 was generated from PBMCs of a healthy donor following two rounds of priming with [Epstein–Barr virus](https://en.wikipedia.org/wiki/Epstein%E2%80%93Barr_virus)-immortalized [B cell](https://en.wikipedia.org/wiki/B_cell) lymphoblastoid JY cells (gift from Gilbert M Lenoir; 48) and subsequent cloning by limited dilution (49). PBMCs were purchased from Stanford Blood Center (Stanford University School of Medicine, Palo Alto, CA). JY-PD-L1 clone 6 was generated by infection of wild-type JY cells with a lentivirus vector pLX-301 encoding human PD-L1 (Millipore Sigma, Saint Louis, MI) in the presence of SureENTRY Transduction Reagent (Qiagen Sciences, MD) for 24 hours followed by selection in 0.2 µg/mL puromycin (Gibco by Life technologies, Grand Island, NY). Puromycin-resistant clone 6 was selected based on high cell surface PD-L1 expression as assessed by flow cytometry. BC4-49, JY-PD-L1, and JY cells were negative for mycoplasma (last tested FEB-2018). Cell line authentication was not conducted.

For the T cell clone coculture activity assay, BC4-49 T cells were harvested on day 7 after antigen re-stimulation, washed twice with PBS, 2 mM EDTA and resuspended as a single cell suspension in Yssel’s medium (Gemini Bioproducts, Sacramento, CA). Test antibodies were 5-fold serially titrated in Yssel’s medium starting from 40 µg/mL. The BC4-49 T cell suspension was added at a density of 2 × 104 cells in 50 µL per well into wells containing 100 µL of titrated antibody in 96 well U bottom tissue culture plates. The antibody/T cell mixture was pre-incubated for 1 hour in an incubator at 37°C with 5% CO2. JY and JY-PD-L1 cells were irradiated in a gamma irradiator at a dose of 5000 Gy, washed twice with PBS, 2 mM EDTA, and then resuspended with Yssel’s medium and filtered through a 40 µm cell strainer (Becton-Dickinson, San Jose, CA) before plating. The JY cell suspension (1 × 104 cells per well) was dispensed into the pre-incubated antibody T cell mixture, at a BC4-49 T cell to JY cell ratio of 2:1. All conditions were performed in duplicate. After a 3-day culture, 100 µL of supernatant per well was harvested for human IFNγ quantification using the Human IFN-gamma Quantikine ELISA kit (R&D Systems, Minneapolis, MN). Statistical analyses were performed using Prism software (GraphPad).

Assessment of cytokine release induced by pembrolizumab

The cytokine levels induced by pembrolizumab were assessed using PBMCs and mobilized antibodies and using whole blood in solution.

PBMCs were isolated from heparinized whole blood obtained from 8 healthy human donors through the MVD (Western Institutional Review Board® Protocol 20121234) under written informed consent. Pembrolizumab and control antibodies were immobilized by coating 50 µL at 0.1 to 1,000 µg/mL onto the walls of wells of 96-well polypropylene plates, and then air-drying overnight. Coated plates were washed 5 times with 200 µL sterile DPBS to remove the salt crystals and unbound antibody. Antibody concentrations cited in data tables assume 100% binding and, therefore, should be considered estimates of concentration. PBMCs (200,000 cells/well) were incubated for 24 hours at 37°C with 5% CO2 in the coated wells containing 200 µL of culture medium and 2% subject-specific plasma. After incubation for 24 hours, cell-conditioned medium was collected, and analyzed for IFNγ, IL-10, IL-12 p70, IL-1β, IL‑2, IL-6, IL-8, and TNF-α using the Meso Scale Discovery Human Proinflammatory Panel 1 V-PLEX kit after diluting 4-fold using Diluent 2, per the manufacturer’s instructions.

The whole blood in vitro cytokine release assay was conducted using blood from 8 human donors through the MVD (Western Institutional Review Board® Protocol 20121234) under written informed consent with similar incubation period and antibody concentrations as in the PBMC assay, but with antibodies in solution phase. Heparinized whole blood was gently mixed and aliquoted into a 96-well V‑bottom 2 mL deep-well plates at 450 µL per well. Dilutions of test and control antibodies were adjusted with Dulbecco's phosphate-buffered saline (DPBS) such that a 50 µL volume added to the 450 µL aliquot of whole blood yielded final antibody concentrations ranging from 0.1 to 1,000 µg/mL. After addition of antibodies, whole blood was gently mixed with a pipette and incubated for 24 hours at 37°C with 5% CO2. Following incubation, plasma was harvested after centrifugation for 15 minutes at 2,000 × g at 4°C, transferred into a non-binding 96-well plate. Cytokine levels in plasma recovered from whole blood incubated with test and control antibodies were measured using Meso Scale Discovery Human Proinflammatory Panel 1 V-PLEX kit with samples processed following the manufacturer's protocol. Briefly, 25 µL of Diluent 2 was added to the wells just prior to the addition of an equal volume of plasma sample (25 µL). The plate was incubated for 2 hours with gentle agitation. Wells were washed 6 times with Wash Buffer, after which 25 µL of Detection Antibody was added into each well. The plate was incubated for 2 with gentle agitation prior to washing plates 6 times with Wash Buffer. This was followed by addition of 150 µL of 2× Reading Buffer into each well. Plates were read using a Meso Scale Discovery plate reader (SECTOR imager SI6000).

In both versions of the cytokine release assay, the effects of pembrolizumab were compared to those from the positive control antibodies, an OKT3 analog muromonab mouse IgG2a (eBioscience), an analog of TGN1412 generated from patent sequences (MSD), and a therapeutic antibody with a known safe clinical profile, Herceptin® trastuzumab (Roche). Six of the 8 donors were tested in both the PBMC and whole blood assays.

Supplementary Data Table S1 and Supplementary Data Table S2 show that the cytokine levels induced by pembrolizumab were low and comparable to those induced by Herceptin® trastuzumab. Supplementary Data Table S1 shows the data from the PBMC/plate-bound antibody assay, while Supplementary Data Table S2 shows the data from the whole blood/antibodies in solution assay.

Evaluation of Antibody-dependent cell-mediated cytotoxicity and Complement-dependent cytotoxicity

Antibody-dependent cell-mediated cytotoxicity (ADCC) assays were conducted using activated human PBMCs as effector cells and activated human CD4+ T cells as target cells. PBMCs were collected through the MVD (Western Institutional Review Board® Protocol 20121234) and under written informed consent. Activated target human CD4+ T cells were incubated with pembrolizumab or control antibodies for 30 minutes at concentrations from 0 to 10 µg/mL. Following incubation, a cell suspension of autologous activated human PBMCs was layered on the activated target human CD4+ T cells at an effector-to-target ratio of 50:1. Cells were incubated for 3 hours, and % specific lysis was determined.

Complement-dependent cytotoxicity (CDC) assays were conducted using activated human CD4+ T cells as target cells and human serum complement as effector molecules. Pembrolizumab and control antibodies were used at concentrations from 0.01 to 10 µg/mL. Activated target human CD4+ T cells were incubated with pembrolizumab or control antibodies for 15 minutes. Human serum complement was added to cells at a 1/6 dilution. Cells were incubated for 4 hours, and % specific lysis was determined.

For both the ADCC and CDC assays, a positive control, anti-human MHC class I antibody (anti-human HLA-ABC clone W6/32, a mouse IgG2a, eBioscience), was included. The negative control antibodies were anti-PCSK9 (human IgG4) (Merck & Co., Inc., Kenilworth, NJ USA) and mouse IgG2a K isotype control clone eBM2a (eBioscience). Statistical analyses were performed using Prism software (GraphPad).

Supplementary Data Figure S2 (ADCC) and Supplementary Data Figure S3 (CDC) show that pembrolizumab does not exhibit ADCC nor CDC activity.

Ex vivo analysis of antigen-specific T cell recall response to viral antigens

The IFN-γ ELISPOT assay was performed to assess the effect of pembrolizumab treatment on overall T-cell response to viral infection. PBMCs were collected at pretreatment; days 3, 8, 15, and 22; and cycle 2, day 1 pre-infusion time points from 17 patients in clinical trial KEYNOTE-001 cohort A under written informed consent. KEYNOTE-001 was conducted in accordance with recognized ethical guidelines and approved by an instititutional review board. Collected blood samples were shipped in sodium heparin vacutainers (BD) at ambient temperature for PBMC isolation and cryo-preservation. Frozen PBMC samples were thawed and analyzed in batches for IFN-γ ELISpot assay. Briefly, 400,000 cells were placed in 96-well ELISPOT plates (Millipore) pre-coated with anti-IFN-γ antibody (Thermo Scientific, clone M700A), incubated in triplicate with CTL-CEF-Class I Peptide Pool “plus” (PanaTecs), a pool of 32 peptides corresponding to major histocompatibility (MHC) Class I-restricted epitopes from Epstein Barr virus, cytomegalovirus, and influenza virus (CEF-32). Cells incubated with phytohemagglutinin A and culture medium were used as positive and negative controls, respectively. After incubation overnight, plates were washed and incubated with biotinylated anti-IFNγ antibody (Thermo Scientific, clone M700A) followed by streptavidin-HRP (BD), visualized by developing spots with 3-amino-9-ethyl-carbazole (Sigma) and hydrogen peroxide. The plates were scanned using a CTL ImmunoSpot® Analyzer and data were processed using SpotMap® software. Statistical analyses were performed using Prism software (GraphPad).

Details of pharmacokinetics of pembrolizumab in cynomolgus monkeys

After a single IV dose of pembrolizumab at 0.3, 3 or 30 mg/kg to cynomolgus monkeys, the decline of serum concentrations of pembrolizumab followed multiphasic kinetics. Data were analyzed using WinNonLin. Slightly greater than dose proportional exposure as judged by AUC0-last was observed between 0.3 and 3.0 mg/kg and approximately dose proportional exposure was observed between 3.0 and 30 mg/kg. Anti-drug antibodies (ADA) were detected in treated animals (all 6 animals in the 0.3 and 3 mg/kg dose groups, and 2 out of 3 animals in the 30 mg/kg dose group). The ADA were detected mostly in the 10-day and 21-day samples after the dosing for the 0.3 mg/kg group and in the 21-day samples for the 3 mg/kg and 30 mg/kg groups. Clearance (CL) appeared to be dose-dependent in the dose range tested with CL of 5.7 ± 0.2, 4.2 ± 0.4 and 3.7 ± 0.1 mL/day per kg for the 0.3, 3, and 30 mg/kg groups, respectively. The terminal half-life (t1/2) was also dose-dependent with longer t1/2 for the 30 mg/kg group (~10 days) than for the 3 and 0.3 mg/kg groups (~4 to 6 days), regardless of the ADA status. The apparent non-linear PK suggests possible target-mediated drug disposition. However, the contribution from ADA cannot be excluded since most of the animals had detectable ADA which may have higher impact at lower dose.

In a GLP one month repeat dose toxicokinetic (TK) study, TK parameters were determined using WinNonLin for the 7-day dosing intervals after the first and the fifth dose. ADA were detected in 7 out of 12 animals in the 6 mg/kg dose group and 1 out of 12 animals in the 40 mg/kg dose group. The ADA positive samples were mostly at 21 days after the first dose for the 6 mg/kg group and at 98 days (recovery period) for the 40 mg/kg dose group. Most ADA positive samples had neutralizing activity, which correlated with significantly lower pembrolizumab concentrations in these animals. The mean t1/2 values in individual ADA-negative animals ranged from 15.7 to 22.3 days across doses. Systemic exposure for the 7-day dosing interval was sex-independent and increased with increasing dose. The Cmax and AUC0-7d were approximately dose proportional after the first dose over the dose range tested. In addition, the systemic exposure over the 7-day dosing interval after the fifth dose was greater than that after the first dose (up to 5.58 fold higher).

In the six month repeat dose study, cynomolgus monkeys (5 males and 5 females) were dosed once every other week via IV administration for a total of 12 doses at 6, 40 or 200 mg/kg of pembrolizumab with a 4 month recovery period. TK parameters were determined using WinNonLin for the 14-day dosing intervals after the first (Day 1), the sixth (Day 71), and the eleventh (Day 141) dose and are shown in Supplementary Data Table S3. ADA were detected in 5 out of 10 animals in the 6 mg/kg dose group during the dosing period at Study Day 43, 71, or 99, which correlated with significantly reduced pembrolizumab concentrations in these animals. No anti- pembrolizumab antibodies were detected in the 40 or 200 mg/kg groups during the dosing phase. T1/2 was evaluated after the last dose (dose 12 at Day 155) from animals that were negative in ADA. The mean t1/2 values ranged from 21 to 22 days across doses after exclusion of ADA positive samples. As with the one month repeat toxicity study, systemic exposure to pembrolizumab was independent of sex. The systemic exposure at Day 71 was similar to Day 141, suggesting that steady state had been achieved by Day 71, which is consistent with approximately 3.5 terminal half-lives of pembrolizumab. Mean AUC0-14d and Cmax values after exclusion of ADA positive animals were approximately dose proportional across all doses on Study Days 1, 71, and 141, with the exception of Study Day 1 for which they were slightly greater than dose proportional in the dose range from 6 to 40 mg/kg. At Day 71, after 6 repeat doses, the systemic exposure over the 14-day dosing interval increased slightly (up to 1.8 fold) as compared to the corresponding values following the first dose.

Following once weekly dosing (one-month study) or once every other week dosing (six-month study) at 6, 40 or 200 mg/kg, systemic exposure was sex-independent and increased with increasing dose. The Cmax and systemic exposure were approximately dose-proportional over the dose range tested after exclusion of ADA positive samples. Repeat dosing resulted in a substantially increased exposure in the one month study and slightly increased exposure in the six month study.

Procedures involving the care and use of animals were reviewed and approved by the Institutional Animal Care and Use Committee at Research Laboratories of Merck & Co., Inc. All toxicology studies were conducted in compliance with Good Laboratory Practice at the Safety Evaluation Center, Schering Plough Research Institute (part of Merck & Co., Inc., Lafayette, New Jersey, USA), Merck & Co., Inc., West Point, Pennsylvania, USA, and Charles River Laboratories, Pathology Associates, Frederick, Maryland, USA.

SUPPLEMENTARY DATA REFERENCES

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SUPPLEMENTARY DATA TABLES

Supplementary Data Table S1 Human PBMC Cytokine Release Assay for Pembrolizumab with Plate-bound Monoclonal Antibodies (median of 8 donors)

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Treatment | Treatment conc. (µg/mL) | IFNγ (pg/mL) | IL-10 (pg/mL) | IL-12 p70 (pg/mL) | IL-1β (pg/mL) | IL-2 (pg/mL) | IL-6 (pg/mL) | IL-8 (pg/mL) | TNF-α (pg/mL) |
| DPBS | 0 | 272.8 | 4.8 | 7.4 | 12.6 | 13.9 | 22.2 | > 2028 | 250.3 |
| Pembrolizumab | 0.1 | 269.7 | 5.4 | 6.7 | 15.8 | 14.4 | 32.5 | > 2028 | 229.4 |
| 1 | 120.9 | 1.6 | 1.2 | 6.5 | 4.0 | 10.7 | > 2028 | 61.2 |
| 10 | 29.0 | 1.1 | 1.6 | 1.9 | 2.6 | 5.2 | 1613.1 | 26.0 |
| 100 | 35.5 | 2.6 | 3.3 | 8.7 | 6.7 | 19.2 | > 2028 | 138.4 |
| 1000 | 40.7 | 4.7 | 5.6 | 38.8 | 11.9 | 109.0 | > 2028 | 512.5 |
| OKT3 analog | 1 | 220.4 | 3.7 | 7.3 | 9.7 | 17.4 | 16.7 | > 2028 | 146.1 |
| 10 | 4607.8 | 52.8 | 14.3 | 67.5 | 174.2 | 147.6 | > 2028 | 1166.2 |
| 100 | 5640.0 | 38.1 | 14.4 | 60.6 | 189.1 | 149.6 | > 2028 | 1264.0 |
| TGN1412 analog | 10 | 345.1 | 12.1 | 4.3 | 5.0 | 333.4 | 16.8 | > 2028 | 276.0 |
| 100 | 307.4 | 16.2 | 9.5 | 40.4 | 497.8 | 110.9 | > 2028 | 877.3 |
| 1000 | 2506.9 | 28.5 | 11.8 | 169.4 | 961.4 | 505.0 | > 2028 | 1264.0 |
| Trastuzumab | 0.1 | 222.2 | 4.7 | 7.5 | 14.5 | 13.7 | 29.7 | > 2028 | 287.7 |
| 1 | 170.8 | 3.0 | 5.2 | 7.0 | 10.2 | 14.4 | > 2028 | 100.8 |
| 10 | 359.0 | 5.1 | 6.7 | 11.7 | 17.5 | 29.9 | > 2028 | 267.1 |
| 100 | 150.1 | 6.5 | 9.3 | 27.7 | 21.9 | 101.3 | > 2028 | 456.2 |
| 1000 | 171.3 | 8.0 | 12.1 | 30.6 | 19.0 | 94.3 | > 2028 | 628.5 |
| Note that monoclonal antibody concentrations listed are approximate and assume that the air-drying method results in 100% monoclonal antibody binding to the plate. DPBS = Dulbecco’s phosphate-buffered saline; IFNγ = interferon gamma; IL = interleukin; PBMC = peripheral blood mononuclear cell; TNF-α = tumor necrosis factor alpha. | | | | | | | | | |

Supplementary Data Table S2 Human Whole Blood Cytokine Release Assay for Pembrolizumab with Monoclonal Antibodies in Solution (median of 8 donors)

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Treatment | Treatment conc. (µg/mL) | IFNγ (pg/mL) | IL-10 (pg/mL) | IL-12 p70 (pg/mL) | IL-1β (pg/mL) | IL-2 (pg/mL) | IL-6 (pg/mL) | IL-8 (pg/mL) | TNF-α (pg/mL) |
| Blood alone | 0 | 16.1 | 0.4 | 0.2 | 0.4 | 0.7 | 1.7 | 187.7 | 1.5 |
| 0 | 15.2 | 0.5 | 0.3 | 0.3 | 0.7 | 2.0 | 204.8 | 1.7 |
| DPBS | 0 | 13.9 | 0.5 | 0.6 | 0.4 | 0.7 | 1.7 | 181.4 | 1.8 |
| Pembrolizumab | 0.1 | 16.1 | 0.4 | 0.2 | 0.4 | 0.7 | 1.9 | 321.0 | 1.8 |
| 1 | 13.8 | 0.5 | 0.2 | 0.3 | 0.7 | 1.8 | 271.8 | 1.7 |
| 10 | 14.0 | 0.5 | 0.2 | 0.4 | 0.7 | 2.0 | 265.1 | 1.9 |
| 100 | 12.0 | 0.4 | 0.4 | 0.5 | 0.7 | 2.1 | 271.8 | 1.8 |
| 1000 | 14.3 | 0.4 | 0.2 | 0.7 | 0.7 | 2.4 | 257.3 | 1.4 |
| OKT3 analog | 100 | 817.3 | 2.8 | 1.3 | 2.1 | 1.7 | 9.2 | 678.4 | 17.9 |
| 400 | 503.4 | 14.2 | 2.7 | 55.6 | 8.1 | 30.1 | 1014.0 | 24.2 |
| TGN1412 analog | 10 | 33.1 | 2.6 | 0.9 | 0.6 | 1.8 | 2.7 | 363.3 | 3.5 |
| 100 | 30.8 | 2.0 | 0.8 | 0.6 | 2.1 | 3.0 | 381.1 | 2.5 |
| 1000 | 26.4 | 2.6 | 1.6 | 1.7 | 2.4 | 8.0 | 1011.0 | 6.3 |
| Trastuzumab | 0.1 | 13.8 | 0.3 | 0.2 | 0.3 | 0.7 | 2.2 | 241.7 | 1.4 |
| 1 | 12.3 | 0.3 | 0.2 | 0.6 | 0.7 | 1.8 | 253.5 | 1.4 |
| 10 | 13.3 | 0.4 | 0.3 | 0.5 | 0.7 | 2.1 | 230.2 | 1.6 |
| 100 | 12.0 | 0.3 | 0.3 | 0.4 | 0.7 | 1.6 | 174.1 | 1.3 |
| 1000 | 8.3 | 0.2 | 0.2 | 0.3 | 0.7 | 1.3 | 128.5 | 0.9 |
| Phosphate-buffered saline was diluted into blood at a concentration equivalent in volume to the amount of other monoclonal antibody additions. DPBS = Dulbecco’s phosphate-buffered saline; IFNγ = interferon gamma; IL = interleukin; TNF-α = tumor necrosis factor alpha. | | | | | | | | | |

Supplementary Data Table S3 Summary of TK Parameters Following Once Every Other Week Repeat Pembrolizumab Dose via IV in Cynomolgus Monkeys (6-month study)

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Dosing day (number of doses)** | **Day 1 (1)** | | | **Day 71 (6)** | | | **Day 141 (11)** | | |
| Dose a (mg/kg) | 6 | 40 | 200 | 6 | 40 | 200 | 6 | 40 | 200 |
| Cmax (µg/mL) b | 174 ± 7.72 | 1840 ± 216 | 7470 ± 529 | 215 ± 43.8 | 1930 ± 93.8 | 10,500 ± 489 | 224 ± 33.9 | 1890 ± 180 | 10,400 ± 772 |
| Tmax (hr) b | 0.33 ± 0.083 | 1.6 ± 0.59 | 4.8 ± 2.2 | 1.3 ± 0.58 | 2.0 ± 0.75 | 0.55 ± 0.12 | 0.25 ± 0.0 | 1.5 ± 0.56 | 1.1 ± 0.55 |
| AUC0-14 days (µg·day/mL) b | 892 ± 34 | 7333 ± 458 | 35333 ± 2517 | 1004 ± 353 | 13042 ± 688 | 64167 ± 2463 | 1200 ± 401 | 13417 ± 1125 | 67500 ± 4458 |
| R c | NA | NA | NA | 1.13 (2.2) | 1.78 | 1.81 | 1.34 (2.69) | 1.83 | 1.91 |
| 1. Number of animals in a dose group : F=5, M=5 2. Arithmetic mean ± standard error of the mean 3. AUC ratio: Day 71 R= AUC0-14days of day 71 interval ÷ AUC0-14days of day 1 interval; Day 141 R= AUC0-14days of day 141 interval ÷ AUC0-14days of day 1 interval.   AUC=area under the concentration curve; Cmax=maximum concentration; hr=hours; NA=not applicable; TK=toxicokinetic; Tmax=time to maximum concentration. | | | | | | | | | |