# Supplementary Methods

## Escalation with overdose control method used to guide dose escalation decisions

After the patients of each cohort completed the observation period (28 days or up to 42 days in case of treatment delays), the logistic regression model was updated with the treatment outcome (i.e., the occurrence of a dose-limiting toxicity) and a new maximum tolerated dose (MTD) estimate was derived. The maximum allowable dose increments were 100%, 67%, and 50% increases from doses of <12 mg/kg, ≥12 to <20 mg/kg, and ≥20 mg/kg, respectively. Dose escalation was stopped if six or more patients were treated at the doses within ±10% of the estimated MTD and the pre-specified precision of the MTD estimate (defined as the lower limit of the 90% credible interval being larger than 50% of the current MTD estimate) was reached. If 12 or more patients were studied within 10% of MTD and the pre-specified precision was not yet reached, escalation was stopped without reaching precision.

## Assessment of PK parameters

To evaluate the accumulation and dose proportionality, a linear mixed effect model was applied to the logarithmically transformed and dose-normalized values of AUC0‑t, Cmin, and Cmax separately for each regimen. Difference in variance was allowed across the dose groups. Patient was fitted as a random effect in the model, dose, time, and their interaction as fixed effects. AUC0‑336h was evaluated for the bi-weekly regimen and AUC0‑168h for the weekly regimen. The dose of 3 mg/kg, for which data from only one patient were available, was excluded from the analysis.

The estimates of the relative change from Day 1 of Cycle 1 to Cycle 2 and to Cycle 4 as well as the estimates of the geometric means at each scheduled time were derived and summarized. Within- and between-patient coefficients of variation (CV%) were derived for each dose and regimen. The estimated geometric means of each dose were compared with the lower reference dose on Day 1 of Cycle 1 and Cycle 4 within each regimen. Dose levels of 6 mg/kg and 12 mg/kg were selected as the reference doses for the bi-weekly and the once weekly regimen, respectively. Dose proportionality was concluded if the 90% confidence intervals for dose-normalized parameters were contained in the 80–125% range of the reference dose.

## Measurement of free and total VEGF-A and Ang-2

Total VEGF-Awas analyzed using an Elecsys® assay (Roche Professional Diagnostics) comprising a biotinylated murine anti-VEGF-A capture antibody (clone 3C5) and a ruthenylated murine anti-VEGF-A detection antibody (clone A4.6.1). Total Ang-2 levels were analyzed using a sandwich-ELISA based test.1 Free VEGF-A and Ang-2 were measured using Quantikine ELISA Kits (R&D Systems Europe, Ltd., Abingdon, UK, Cat. No. DVE00 and DANG20, respectively). Drug-bound Ang-2 was removed as described previously.2

## References

1. Babitzki G, Hidalgo M, Massard C, Martinez Garcia M, Le Tourneau C, Boni V, et al. Biomarkers related to Vanucizumab single agent therapy in serial plasma, tumor tissue and skin wound-healing biopsies of patients with advanced solid tumors. Poster presented at the 2015 ESMO Annual Meeting, Vienna, Austria, September 25-29, 2015

2. Stubenrauch K, Wessels U, Essig U, Vogel R, Waltenberger H, Hanbauer A, et al: An immunodepletion procedure advances free angiopoietin-2 determination in human plasma samples during anti-cancer therapy with bispecific anti-Ang2/VEGF CrossMab. J Pharm Biomed Anal 2015; 102:459-467.

**Supplementary Table S1.** Criteria for defining dose-limiting toxicities.

|  |  |  |
| --- | --- | --- |
| **SOC disorder** | **CTCAE term** | **DLT criteria** |
| General and administration  site conditions | * Fatigue | * G ≥3 for > 7 consecutive days |
| Blood and lymphatic | * Febrile neutropenia | * G ≥3 i.e., ANC <1.0 x 109/L, associated w/ single BT >38.3°C or sustained BT ≥38°C for >1 hr |
| Vascular | * Hypertension | * G 3, i.e., BP ≥160/100 mmHg for >14 consecutive days, despite appropriate anti-hypertensive medication * G 4 |
| * Thromboembolic event | * G 2, requiring full dose anti-coagulant therapy for >14 consecutive days * G ≥3 |
| Gastrointestinal | * GI- Perforation | * G ≥1 |
| * Diarrhea | * G ≥ 3 for >48 hrs, despite the use of anti-diarrhea therapy |
| * Nausea/ vomiting | * G ≥3 for >48 hrs, despite the use of anti-emetic therapy |
| Respiratory, thoracic and mediastinal | * Brochopulmonary hemorrhage | * G 1 for >14 consecutive days * G ≥2 |
| Nervous system | * Intracranial hemorrhage | * G ≥1 |
| * RPLS | * G ≥1 |
| Renal and urinary | * Proteinuria | * Proteinuria ≥2 g/24 hrs for >14 daysa * G ≥3 (urinary protein ≥3.5 g/24 hrs) |
| Investigations | * Platelet count decreased (Thrombocytopenia) | * G 3 for >7 consecutive days and/or with signs of bleeding * G 4 |
| * Neutrophil count decreased | * G 4 |
| Other hematologic & non-hematologic disorders | * Any fistula formation involving an internal organ | * G ≥1 |
| * Other adverse event | Any other treatment-related G ≥3 toxicity except:   * Investigations (e.g., laboratory values and non-fluid associated netto body weight gain) G ≥3 which are judged not clinically significant by the Investigator * Skin toxicity G ≥3 in the absence of adequate supportive care * Infusion-related / hypersensitivity reactions * Alopecia (any grade) |
| **Other** | **DLT Criteria** | |
| Skipped/delayed dose | * Delay in 2nd cycle administration of vanucizumab for >14 days due to failure of recovery from study drug related adverse event(s) | |

Abbreviations: ANC, absolute neutrophil count; BP, blood pressure; BT, body temperature; CTCAE, Common Terminology Criteria for Adverse Events, DLT, dose-limiting toxicity; G, grade (According to CTCAE version 4.03); GI, gastrointestinal; RPLS, Reversible posterior leukoencephalopathy syndrome; SOC, system organ class.

aNot according to CTCAEv4.03 or later.

**Supplementary Table S2.** Plasma pharmacokinetic parameters of vanucizumab on Cycles 1 and 4 following bi-weekly administration of ascending doses of vanucizumab.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Dose [mg/kg]** | **Cycle1** | | | | | | | | **Cycle 4** | | | | | | | | | |
| **3** | **6** | | **12** | | | **19** | **30** | **3** | | **6** | | **12** | | **19** | | **30** | |
| **tmax [h]** |  |  | |  | | |  |  |  | |  | |  | |  | |  | |
| N | 1 | 3 | | 4 | | | 7 | 6 | 1 | | 1 | | 2 | | 5 | | 4 | |
| Median | − | 2.00 | | 1.75 | | | 2.03 | 2.0 | − | | − | | − | | 2.0 | | 2.02 | |
| Min−Max | 7.5\* | 1.5−2.0 | | 1.5−2.07 | | | 1.67−23.9 | 1.53−6.0 | 2.0a | | 0.5a | | 2.05; 4.0 a | | 0.67−6.0 | | 2.0−6.5 | |
| **Cmax [μg/mL]** |  |  | |  | | |  |  |  | |  | |  | |  | |  | |
| N | 1 | 3 | | 4 | | | 7 | 6 | 1 | | 1 | | 2 | | 5 | | 4 | |
| GM | 91.6\* | 177 | | 258 | | | 518 | 648 | 143a | | 175a | | 363; 464a | | 676 | | 983 | |
| CV% GM | − | 8.50 | | 22.8 | | | 28.0 | 29.4 | − | | − | | − | | 14.0 | | 39.0 | |
| **AUC0-τ [μg•h/mL]** |  | |  | |  | |  |  |  | |  | |  | |  | |  | |
| N | 1 | | 3 | | 4 | | 7 | 6 | 1 | | 1 | | 2 | | 5 | | 4 | |
| GM | 12400\* | | 18700 | | 33300 | | 58600 | 98800 | 20100a | | 22700a | | 61400; 562000a | | 112000 | | 153000 | |
| CV% GM | − | | 3.04 | | 22.9 | | 13.5 | 38.2 | − | | − | | − | | 26.5 | | 35.2 | |
| **t1/2 [h]** |  | |  | | |  |  |  |  |  | |  | |  | |  | |
| N | − | | − | | | − | − | − | 1 | 1 | | 2 | | 4 | | 4 | |
| GM | − | | − | | | − | − | − | 176a | 149a | | 334; 167a | | 222 | | 208 | |
| CV% GM | − | | − | | | − | − | − | − | − | | − | | 37.7 | | 15.8 | |
| **CL [mL/h]** |  | |  | | |  |  |  |  |  | |  | |  | |  | |
| N | − | | − | | | − | − | − | 1 | 1 | | 2 | | 4 | | 4 | |
| GM | − | | − | | | − | − | − | 10.9a | 15.9a | | 10.2; 18.0a | | 13.6 | | 14.7 | |
| CV% GM | − | | − | | | − | − | − | − | − | | − | | 33.8 | | 14.0 | |
| **Vss [mL]** |  | |  | | |  |  |  |  |  | |  | |  | |  | |
| N | − | | − | | | − | − | − | 1 | 1 | | 2 | | 4 | | 4 | |
| GM | − | | − | | | − | − | − | 2610a | 3170a | | 4360; 3750a | | 3960 | | 4040 | |
| CV% GM | − | | − | | | − | − | − | − | − | | − | | 35.9 | | 26.8 | |
| **Cmin [μg/mL]** |  | |  | | |  |  |  |  |  | |  | |  | |  | |
| N | 1 | | 3 | | | 4 | 7 | 6 | 1 | 1 | | 2 | | 5 | | 4 | |
| GM | 17.2\* | | 21.0 | | | 42.2 | 90.4 | 132 | 29.3a | 28.8a | | 117; 70.3a | | 173 | | 247 | |
| CV% GM | − | | 19.5 | | | 49.2 | 10.9 | 42.7 | − | − | | − | | 37.5 | | 32.8 | |
| **RA(AUC) [ − ]** |  | |  | | |  |  |  |  |  | |  | |  | |  | |
| N | − | | − | | | − | − | − | 1 | 1 | | 2 | | 5 | | 4 | |
| GM | − | | − | | | − | − | − | 1.62a | 1.24a | | 1.64; 1.41a | | 1.88 | | 1.57 | |
| CV% GM | − | | − | | | − | − | − | − | − | | − | | 22.1 | | 16.5 | |
| **RA(Cmin) [ − ]** |  | |  | | |  |  |  |  |  | |  | |  | |  | |
| N | − | | − | | | − | − | − | 1 | 1 | | 2 | | 5 | | 4 | |
| GM | − | | − | | | − | − | − | 1.70a | 1.28a | | 1.90; 1.29a | | 1.93 | | 1.89 | |
| CV% GM | − | | − | | | − | − | − | − | − | | − | | 34.4 | | 22.3 | |

Abbreviations: AUC0-τ , area under the concentration-time curve from 0 to τ (336 h); CL, clearance; Cmax, maximum concentration; Cmin, minimum concentration; CV, coefficient of variation; GM, geometric mean; RA(AUC), accumulation ratio based on AUC and relative to Cycle 1; RA(Cmin), accumulation ratio based on Cmin and relative to Cycle 1; t1/2, half-life; Tmax, time to peak concentration; Vss, volume of distribution at steady state.

aIndividual value is reported.

**Supplementary Table S3.** Plasma pharmacokinetic parameters of vanucizumab on Cycles 1 and 4 following weekly administration of ascending doses of vanucizumab.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Dose [mg/kg]** | **Cycle1** | | | **Cycle 4** | | |
| **10** | **20** | **30** | **10** | **20** | **30** |
| **tmax [h]** |  |  |  |  |  |  |
| N | 4 | 4 | 12 | 4 | 4 | 10 |
| Median | 3.0 | 6.0 | 2.0 | 1.38 | 4.75 | 2.0 |
| Min−Max | 2.0−6.0 | 2.0−6.0 | 1.5−4.0 | 0.55−6.0 | 2.0−6.0 | 0.5−6.05 |
| **Cmax [μg/mL]** |  |  |  |  |  |  |
| N | 4 | 4 | 12 | 4 | 4 | 10 |
| GM | 283 | 451 | 649 | 488 | 854 | 1160 |
| CV% GM | 26.6 | 27.5 | 17.6 | 44.5 | 18.5 | 27.3 |
| **AUC0-τ [μg•h/mL]** |  |  |  |  |  |  |
| N | 4 | 4 | 12 | 3 | 3 | 9 |
| GM | 27200 | 39900 | 58800 | 43200 | 85800 | 131000 |
| CV% GM | 45.1 | 21.9 | 25.7 | 60.2 | 7.86 | 40.7 |
| **t1/2 [h]** |  |  |  |  |  |  |
| N | − | − | − | 3 | 3 | 8 |
| GM | − | − | − | 151 | 170 | 174 |
| CV% GM | − | − | − | 150 | 15.4 | 31.0 |
| **CL [mL/h]** |  |  |  |  |  |  |
| N | − | − | − | 3 | 3 | 8 |
| GM | − | − | − | 20.1 | 14.4 | 15.3 |
| CV% GM | − | − | − | 68.0 | 14.9 | 34.2 |
| **Vss [mL]** |  |  |  |  |  |  |
| N | − | − | − | 3 | 3 | 8 |
| GM | − | − | − | 3910 | 3290 | 3660 |
| CV% GM | − | − | − | 79.1 | 33.4 | 21.7 |
| **Cmin [ug/mL]** |  |  |  |  |  |  |
| N | 4 | 4 | 12 | 4 | 4 | 10 |
| GM | 66.8 | 129 | 220 | 135 | 374 | 484 |
| CV% GM | 44.2 | 35.9 | 32.1 | 128 | 13.5 | 53.3 |
| **RA(AUC) [−]** |  |  |  |  |  |  |
| N | − | − | − | 3 | 3 | 9 |
| GM | − | − | − | 1.33 | 2.38 | 2.23 |
| CV% GM | − | − | − | 41.7 | 2.16 | 21.3 |
| **RA(Cmin) [−]** |  |  |  |  |  |  |
| N | − | − | − | 4 | 4 | 10 |
| GM | − | − | − | 2.02 | 2.89 | 2.24 |
| CV% GM | − | − | − | 118 | 30.7 | 33.4 |

Abbreviations: AUC0-τ , area under the concentration-time curve from 0 to τ (336 h); CL, clearance; Cmax, maximum concentration; Cmin, minimum concentration; CV, coefficient of variation; GM, geometric mean; RA(AUC), accumulation ratio based on AUC and relative to Cycle 1; RA(Cmin), accumulation ratio based on Cmin and relative to Cycle 1; t1/2, half-life; Tmax, time to peak concentration; Vss, volume of distribution at steady state.

**Supplementary Figure S1.** Parametric KTRANS map for a patient with colorectal carcinoma treated with 30 mg/kg vanucizumab bi-weekly.

