

## Supplementary Materials

### Short-term study on in-use stability of opened bevacizumab biosimilar PF-06439535 vials

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**Table S1** Overview of the area percent determined for the side peaks observed in PF-06439535 via SEC after storage at 2–8°C at different timepoints

| Timepoint at 2–8°C | Sample # | Side peak 1 (RT about 7.7 min) |        | Side peak 2 (RT about 8.1 min) |        | Side peak 3 (RT about 12.2 min) |        |
|--------------------|----------|--------------------------------|--------|--------------------------------|--------|---------------------------------|--------|
|                    |          | Area %                         | Mean % | Area %                         | Mean % | Area %                          | Mean % |
| D0                 | Vial 1   | 0.057                          | 0.056  | 2.791                          | 2.829  | 0.538                           | 0.630  |
|                    | Vial 1   | 0.060                          |        | 2.836                          |        | 0.669                           |        |
|                    | Vial 1   | 0.051                          |        | 2.858                          |        | 0.682                           |        |
|                    | Vial 2   | 0.062                          | 0.060  | 2.860                          | 2.872  | 0.691                           | 0.642  |
|                    | Vial 2   | 0.058                          |        | 2.876                          |        | 0.548                           |        |
|                    | Vial 2   | 0.061                          |        | 2.880                          |        | 0.688                           |        |
|                    | Vial 3   | 0.060                          | 0.060  | 2.908                          | 2.906  | 0.683                           | 0.632  |
|                    | Vial 3   | 0.058                          |        | 2.896                          |        | 0.537                           |        |
| Vial 3             | 0.062    | 2.914                          |        | 0.676                          |        |                                 |        |
| D2                 | Vial 1   | 0.068                          | 0.063  | 3.014                          | 3.023  | 1.495                           | 1.413  |
|                    | Vial 1   | 0.059                          |        | 3.031                          |        | 1.386                           |        |
|                    | Vial 1   | 0.062                          |        | 3.025                          |        | 1.357                           |        |
|                    | Vial 2   | 0.056                          | 0.058  | 3.034                          | 3.032  | 1.539                           | 1.482  |
|                    | Vial 2   | 0.060                          |        | 3.032                          |        | 1.406                           |        |
|                    | Vial 2   | 0.058                          |        | 3.031                          |        | 1.500                           |        |
|                    | Vial 3   | 0.063                          | 0.062  | 3.034                          | 3.039  | 1.474                           | 1.452  |
|                    | Vial 3   | 0.061                          |        | 3.053                          |        | 1.538                           |        |
| Vial 3             | 0.062    | 3.030                          |        | 1.345                          |        |                                 |        |
| D4                 | Vial 1   | 0.045                          | 0.050  | 2.856                          | 2.872  | 1.400                           | 1.396  |
|                    | Vial 1   | 0.056                          |        | 2.869                          |        | 1.421                           |        |
|                    | Vial 1   | 0.050                          |        | 2.891                          |        | 1.367                           |        |
|                    | Vial 2   | 0.047                          | 0.050  | 2.883                          | 2.887  | 1.383                           | 1.374  |
|                    | Vial 2   | 0.051                          |        | 2.879                          |        | 1.358                           |        |
|                    | Vial 2   | 0.051                          |        | 2.898                          |        | 1.381                           |        |
|                    | Vial 3   | 0.047                          | 0.049  | 2.896                          | 2.903  | 1.360                           | 1.371  |
|                    | Vial 3   | 0.052                          |        | 2.907                          |        | 1.392                           |        |
| Vial 3             | 0.047    | 2.905                          |        | 1.362                          |        |                                 |        |
| D8                 | Vial 1   | 0.057                          | 0.049  | 2.877                          | 2.890  | 1.391                           | 1.466  |
|                    | Vial 1   | 0.045                          |        | 2.895                          |        | 1.466                           |        |
|                    | Vial 1   | 0.044                          |        | 2.897                          |        | 1.542                           |        |
|                    | Vial 2   | 0.047                          | 0.043  | 2.893                          | 2.908  | 1.405                           | 1.403  |
|                    | Vial 2   | 0.039                          |        | 2.916                          |        | 1.444                           |        |
|                    | Vial 2   | 0.042                          |        | 2.915                          |        | 1.361                           |        |
|                    | Vial 3   | 0.041                          | 0.044  | 2.927                          | 2.919  | 1.425                           | 1.435  |
|                    | Vial 3   | 0.043                          |        | 2.901                          |        | 1.404                           |        |
| Vial 3             | 0.048    | 2.930                          |        | 1.475                          |        |                                 |        |

Area % represents the individual area percentage of the respective side peak compared to the bevacizumab PF-06439535 peak. Mean % represents the average individual area percentage of the respective side peak compared to the PF-06439535 peak based on triplicate measurement.

D0, day 0 (start); D2, day 2; D4, day 4; D8, day 8; RT, retention time; SEC, size exclusion chromatography.

**Table S2** Overview of the area percent determined for the side peaks observed in PF-06439535 via SEC after storage at 25°C at different timepoints

| Timepoint at 25°C | Sample #     | Side peak 1 (RT about 7.7 min) |        | Side peak 2 (RT about 8.1 min) |        | Side peak 3 (RT about 12.2 min) |        |
|-------------------|--------------|--------------------------------|--------|--------------------------------|--------|---------------------------------|--------|
|                   |              | Area %                         | Mean % | Area %                         | Mean % | Area %                          | Mean % |
| D0                | See table S1 |                                |        |                                |        |                                 |        |
| D2                | Vial 1       | 0.049                          | 0.053  | 2.937                          | 2.984  | 1.446                           | 1.432  |
|                   | Vial 1       | 0.051                          |        | 3.004                          |        | 1.417                           |        |
|                   | Vial 1       | 0.058                          |        | 3.010                          |        | 1.433                           |        |
|                   | Vial 2       | 0.056                          | 0.050  | 3.016                          | 3.015  | 1.419                           | 1.431  |
|                   |              | 0.050                          |        | 3.013                          |        | 1.398                           |        |
|                   |              | 0.044                          |        | 3.015                          |        | 1.476                           |        |
|                   | Vial 3       | 0.053                          | 0.053  | 3.009                          | 3.011  | 1.406                           | 1.444  |
|                   |              | 0.054                          |        | 3.012                          |        | 1.521                           |        |
|                   |              | 0.051                          |        | 3.013                          |        | 1.406                           |        |
| D4                | Vial 1       | 0.047                          | 0.052  | 2.923                          | 2.922  | 1.315                           | 1.364  |
|                   | Vial 1       | 0.056                          |        | 2.919                          |        | 1.378                           |        |
|                   | Vial 1       | 0.053                          |        | 2.924                          |        | 1.400                           |        |
|                   | Vial 2       | 0.055                          | 0.052  | 2.941                          | 2.948  | 1.351                           | 1.346  |
|                   |              | 0.054                          |        | 2.947                          |        | 1.352                           |        |
|                   |              | 0.047                          |        | 2.957                          |        | 1.335                           |        |
|                   | Vial 3       | 0.049                          | 0.051  | 2.950                          | 2.950  | 1.359                           | 1.354  |
|                   |              | 0.052                          |        | 2.952                          |        | 1.369                           |        |
|                   |              | 0.052                          |        | 2.947                          |        | 1.334                           |        |
| D8                | Vial 1       | 0.047                          | 0.044  | 2.988                          | 2.984  | 1.306                           | 1.284  |
|                   | Vial 1       | 0.039                          |        | 2.988                          |        | 1.203                           |        |
|                   | Vial 1       | 0.045                          |        | 2.975                          |        | 1.344                           |        |
|                   | Vial 2       | 0.048                          | 0.053  | 2.980                          | 2.986  | 1.356                           | 1.360  |
|                   |              | 0.056                          |        | 2.975                          |        | 1.373                           |        |
|                   |              | 0.054                          |        | 3.004                          |        | 1.350                           |        |
|                   | Vial 3       | 0.049                          | 0.048  | 3.004                          | 3.008  | 1.323                           | 1.332  |
|                   |              | 0.047                          |        | 3.007                          |        | 1.328                           |        |
|                   |              | 0.049                          |        | 3.014                          |        | 1.346                           |        |

Area % represents the individual area percentage of the respective side peak compared to the bevacizumab PF-06439535 peak. Mean % represents the average individual area percentage of the respective side peak compared to the PF-06439535 peak based on triplicate measurement.

D0, day 0 (start); D2, day 2; D4, day 4; D8, day 8; RT, retention time; SEC, size exclusion chromatography.

**Table S3** Overview of the area percent determined for the side peaks observed in PF-06439535 via SEC when stored unopen at 2–8°C and 25°C

| Timepoint   | Sample # | Side peak 1<br>(RT about 7.7 min) |        | Side peak 2<br>(RT about 8.1 min) |        | Side peak 3<br>(RT about 12.2 min) |        |
|-------------|----------|-----------------------------------|--------|-----------------------------------|--------|------------------------------------|--------|
|             |          | Area %                            | Mean % | Area %                            | Mean % | Area %                             | Mean % |
| D8<br>2–8°C | Vial 1   | 0.043                             | 0.043  | 2.907                             | 2.916  | 1.453                              | 1.439  |
|             | Vial 1   | 0.044                             |        | 2.905                             |        | 1.425                              |        |
|             | Vial 1   | 0.043                             |        | 2.935                             |        | 1.439                              |        |
|             | Vial 2   | 0.039                             | 0.041  | 2.930                             | 2.939  | 1.435                              | 1.448  |
|             | Vial 2   | 0.042                             |        | 2.953                             |        | 1.352                              |        |
|             | Vial 2   | 0.042                             |        | 2.935                             |        | 1.558                              |        |
|             | Vial 3   | 0.042                             | 0.045  | 2.903                             | 2.915  | 1.440                              | 1.463  |
|             | Vial 3   | 0.053                             |        | 2.911                             |        | 1.439                              |        |
|             | Vial 3   | 0.041                             |        | 2.930                             |        | 1.510                              |        |
| D8<br>25°C  | Vial 1   | 0.046                             | 0.046  | 3.003                             | 3.034  | 1.326                              | 1.342  |
|             | Vial 1   | 0.043                             |        | 3.059                             |        | 1.343                              |        |
|             | Vial 1   | 0.050                             |        | 3.040                             |        | 1.357                              |        |
|             | Vial 2   | 0.048                             | 0.050  | 3.019                             | 3.005  | 1.307                              | 1.363  |
|             | Vial 2   | 0.054                             |        | 3.011                             |        | 1.317                              |        |
|             | Vial 2   | 0.048                             |        | 2.984                             |        | 1.466                              |        |
|             | Vial 3   | 0.050                             | 0.048  | 3.006                             | 3.013  | 1.329                              | 1.377  |
|             | Vial 3   | 0.049                             |        | 3.021                             |        | 1.415                              |        |
|             | Vial 3   | 0.044                             |        | 3.013                             |        | 1.388                              |        |

Area % represents the individual area percentage of the respective side peak compared to the bevacizumab PF-06439535 peak. Mean % represents the average individual area percentage of the respective side peak compared to the PF-06439535 peak based on triplicate measurement.

D8, day 8; RT, retention time; SEC, size exclusion chromatography.

**Table S4** Overview on PF-06439535 sample dilutions tested for linearity of the SEC method and the corresponding peak areas\*

| Concentration of PF-06439535 sample solution (mg/mL) | Peak area   |
|--|-------------|
| 0.5  | 21,173,353  |
|  | 21,532,498  |
|  | 21,352,926  |
| 1  | 42,239,922  |
|  | 42,634,521  |
|  | 42,437,222  |
| 2  | 82,330,125  |
|  | 82,867,169  |
|  | 82,598,647  |
| 4  | 140,603,960 |
|  | 141,365,353 |
|  | 140,984,657 |
| 5  | 160,849,579 |
|  | 161,323,314 |
|  | 161,086,447 |

\*The corresponding calibration line is presented in figure S1.

SEC, size exclusion chromatography.

**Table S5** Overview on the results obtained for precision of SEC

| PF-06439535 concentration | Peak area  |             |
|---------------------------|------------|-------------|
|                           | 0.5 mg/mL  | 5.0 mg/mL   |
| Sample                    |            |             |
| 1                         | 22,558,035 | 184,282,430 |
| 2                         | 22,653,565 | 184,445,365 |
| 3                         | 22,604,336 | 184,165,762 |
| 4                         | 22,569,942 | 184,424,478 |
| 5                         | 22,559,188 | 184,638,158 |
| 6                         | 22,558,396 | 184,391,471 |
| Mean                      | 22,583,910 | 184,391,277 |
| Standard deviation        | 38,459.856 | 159,801.607 |
| Variation coefficient (%) | 0.17       | 0.09        |

SEC, size exclusion chromatography.

**Table S6** Overview on the results obtained for accuracy of the SEC method\*

|                           | Molecular weight |           |           |           |           |
|---------------------------|------------------|-----------|-----------|-----------|-----------|
| PF-06439535 concentration | 0.5 mg/mL        | 1.0 mg/mL | 2.0 mg/mL | 4.0 mg/mL | 5.0 mg/mL |
| Sample                    |                  |           |           |           |           |
| 1                         | 149,000          | 147,477   | 147,477   | 145,969   | 150,537   |
| 2                         | 147,477          | 145,969   | 147,477   | 149,000   | 150,537   |
| Mean                      | 148,239          | 146,723   | 147,477   | 147,485   | 150,537   |

\*The molecular weight determined from different bevacizumab PF-06439535 sample dilutions was compared with the declared molecular weight of bevacizumab corresponding to 149 kDa.

SEC, size exclusion chromatography.

**Figure S1** Calibration line corresponding to table S4