Tumor cell PD-L1 expression is a strong predictor of unfavorable prognosis in immune checkpoint therapy-naive clear cell renal cell cancer

Katharina Möller¹, Christoph Fraune¹, Niclas C Blessin¹, Maximilian Lennartz¹, Martina Kluth¹, Claudia Hube-Magg¹, Linnea Lindhorst¹, Roland Dahlem², Margit Fisch², Till Eichenauer², Silke Riechardt², Ronald Simon¹#, Guido Sauter¹, Franziska Büscheck¹, Wolfgang Höppner³, Cord Matthies⁴, Ousman Doh⁵, Till Krech¹,6, Andreas H Marx¹,7, Henrik Zecha⁸, Michael Rink², Stefan Steurer¹, Till S Clauditz¹

¹Institute of Pathology, University Medical Center Hamburg-Eppendorf, Hamburg, German
²Department of Urology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
³Department of Urology, Itzehoe Medical Center, Itzehoe, Germany.
⁴Department of Urology, Bundeswehr Hospital Hamburg, Hamburg, Germany
⁵Department of Urology, Regio Medical Center Elmshorn, Elmshorn, Germany
⁶Institute of Pathology, Clinical Center Osnabrueck, Osnabrück, Germany
⁷Department of Pathology, Academic Hospital Fuerth, Fuerth, Germany
⁸Department of Urology, Albertinen Clinic, Hamburg, Germany

# Corresponding author: Dr. Ronald Simon, Institute of Pathology, University Medical Center Hamburg-Eppendorf, Martinistr. 52, 20246 Hamburg, Germany, Tel: +49 40 7410 57214, FAX +49 40 7410 55997, E-mail: R.Simon@uke.de
## Supplementary Table 1. PD-L1 expression in tumor cells and immune cells and histotype

| Histotype                                      | PD-L1 in cancer cells | PD-L1 in immune cells |
|------------------------------------------------|-----------------------|-----------------------|
|                                                | n         | PD-L1 positive cut-off | p vs clear cell cancer | PD-L1 positive cut-off | p vs clear cell cancer | PD-L1 positive cut-off | p vs clear cell cancer | PD-L1 positive cut-off | p vs clear cell cancer | none | few | medium | many | p vs clear cell cancer |
| clear cell renal cell carcinoma             | 633       | 6.3                  | 6.3                   | 5.1                   | 2.4                   | 92.3                  | 4.7                  | 2.5                  | 0.5                   | 1%    |     |        |      |                          |
| papillary renal cell carcinoma          | 165       | 18.2                 | <0.0001               | 18.2                  | <0.0001               | 6.1                   | 0.0252               | 86.1                 | 8.5                  | 3     | 2.4 | 0.0483 |      |                          |
| oncocytoma                                   | 103       | 41.7                 | <0.0001               | 41.7                  | <0.0001               | 13.6                  | <0.0001              | 94.2                 | 3.9                  | 1.9 | 0    | 0.7484 |      |                          |
| chromophobe renal cell carcinoma        | 64        | 18.8                 | 0.0017                | 18.8                  | 0.0017                | 9.4                   | 0.0095               | 100                  | 0                    | 0    | 0    | 0.0203 |      |                          |
| clear cell (tubulo) papillary renal cell carcinoma | 15 | 0                    | 0                     | 0                     | 0                     | 93.3                  | 0                    | 6.7                  | 0                    |      |     |        |      |                          |
| nephroblastoma                               | 17        | 5.9                  | 5.9                   | 5.9                   | 0                     | 82.4                  | 17.6                 | 0                    | 0                    |      |     |        |      |                          |
| Xp11.2 translocation renal cell carcinoma | 7         | 14.3                 | 14.3                  | 14.3                  | 14.3                  | 100                   | 0                    | 0                    | 0                    |      |     |        |      |                          |
| collecting duct carcinoma                  | 4         | 0                    | 0                     | 0                     | 0                     | 100                   | 0                    | 0                    | 0                    |      |     |        |      |                          |
| metanephric adenoma                         | 4         | 0                    | 0                     | 0                     | 0                     | 100                   | 0                    | 0                    | 0                    |      |     |        |      |                          |
| tubulocystic renal cell carcinoma          | 2         | 0                    | 0                     | 0                     | 0                     | 0                     | 0                    | 0                    | 0                    |      |     |        |      |                          |
| multilocular cystic clear cell renal cell neoplasm of low malignancy | 2 | 0 | 0 | 0 | 0 | 100 | 0 | 0 | 0 |      |     |        |      |                          |
| medullary carcinoma                         | 1         | 0                    | 0                     | 0                     | 0                     | 100                   | 0                    | 0                    | 0                    |      |     |        |      |                          |
| neuroendocrine carcinoma                   | 1         | 0                    | 0                     | 0                     | 0                     | 100                   | 0                    | 0                    | 0                    |      |     |        |      |                          |
| carcinoma no otherwise specified (NOS)     | 15        | 40                   | 40                    | 40                    | 26.7                  | 80                    | 6.7                  | 6.7                  | 6.7                  |      |     |        |      |                          |
Supplementary Table 2. PD-L1 expression in cancer cells and immune cells and phenotype of papillary RCC

|                  | PD-L1 in cancer cells | PD-L1 in immune cells |
|------------------|-----------------------|-----------------------|
|                  | n                     | PD-L1 positive cut-off| p value  | PD-L1 positive cut-off| p value  | PD-L1 positive cut-off| p value  | PD-L1 positive cut-off| p value  | PD-L1 positive cut-off| p value  | PD-L1 positive cut-off| p value  | PD-L1 positive cut-off| p value  | PD-L1 positive cut-off| p value  |
| papillary cancers| 165                   | 18.2                  | 18.2     | 17.0     | 6.1      | 86.1 | 8.5 | 3.0 | 2.4 |
| ISUP             |                       |                       |          |          |          |     |     |     |     |
| 1                | 35                    | 14.3                  | 0.4315   | 14.3     | 0.4315  | 14.3 | 0.5070 | 5.7 | 0.5987 | 88.6 | 8.6 | 2.9 | 0.0 | 0.9644 |
| 2                | 76                    | 15.8                  | 0.4315   | 14.5     | 0.4315  | 14.5 | 4.0  | 85.5 | 7.9   | 4.0  | 2.6 |
| 3                | 51                    | 25.5                  | 14.3     | 25.5     | 0.4315  | 23.5 | 9.8  | 84.3 | 9.8   | 2.0  | 3.9 |
| 4                | 1                     | 0.0                   | 0.0      | 0.0      | 0.0     | 0.0  | 100.0 | 0.0 | 0.0   | 0.0  | 0.0 |
| Fuhrmann         |                       |                       |          |          |          |     |     |     |     |
| 1                | 1                     | 0.0                   | 0.0      | 0.0      | 0.0     | 0.0  | 100.0 | 0.0 | 0.0   | 0.0  | 0.0 | 0.0 | 0.0 | 0.8111 |
| 2                | 112                   | 14.3                  | 0.6115   | 14.3     | 0.6115  | 14.3 | 3.6  | 86.6 | 8.0   | 3.6  | 1.8 |
| 3                | 47                    | 27.7                  | 27.7     | 25.5     | 0.6115  | 25.5 | 10.6 | 85.1 | 10.6  | 2.1  | 2.1 |
| 4                | 3                     | 33.3                  | 33.3     | 33.3     | 33.3    | 33.3 | 66.7 | 0.0  | 0.0   | 33.3 | 0.0 |
| Thoenes          |                       |                       |          |          |          |     |     |     |     |
| 1                | 44                    | 13.6                  | 0.6115   | 13.6     | 0.6115  | 13.6 | 2.3  | 88.6 | 6.8   | 2.3  | 2.3 | 0.6146 |
| 2                | 110                   | 20.0                  | 20.0     | 18.2     | 0.6115  | 18.2 | 7.3  | 84.6 | 10.0  | 3.6  | 1.8 |
| 3                | 9                     | 22.2                  | 22.2     | 22.2     | 0.6115  | 22.2 | 11.1 | 88.9 | 0.0   | 0.0  | 11.1 |
| UICC             |                       |                       |          |          |          |     |     |     |     |
| 1                | 94                    | 20.2                  | 0.6597   | 20.2     | 0.6597  | 20.2 | 5.3  | 85.1 | 8.5   | 4.3  | 2.1 | 0.7294 |
| 2                | 15                    | 13.3                  | 13.3     | 13.3     | 0.6597  | 13.3 | 0.0  | 86.7 | 6.7   | 0.0  | 6.7 |
| 3                | 3                     | 33.3                  | 33.3     | 33.3     | 0.6597  | 33.3 | 100.0 | 0.0 | 0.0   | 0.0  | 0.0 |
| 4                | 9                     | 33.3                  | 33.3     | 33.3     | 0.6597  | 33.3 | 88.9 | 0.0  | 0.0   | 0.0  | 11.1 |
| tumor stage      |                       |                       |          |          |          |     |     |     |     |
| pT1              | 117                   | 18.8                  | 0.4465   | 18.8     | 0.4465  | 17.1 | 6.0  | 0.6808 | 87.2 | 7.7 | 3.4 | 1.7 | 0.8090 |
| pT2              | 32                    | 12.5                  | 12.5     | 12.5     | 0.4465  | 12.5 | 3.1  | 81.3 | 9.4   | 3.1  | 6.3 |
| pT3-4            | 10                    | 30.0                  | 30.0     | 30.0     | 0.4465  | 30.0 | 10.0 | 90.0 | 10.0  | 0.0  | 0.0 |
| lymph node metastasis | 0 | 14 | 14.3 | 0.4419 | 14.3 | 0.4419 | 14.3 | 0.4675 | 7.1 | 0.6087 | 85.7 | 7.1 | 0.0 | 7.1 | 0.5926 |
|----------------------|--|--|--|--|--|--|--|--|--|--|--|--|--|--|---|
| ≥1                   | 7 | 28.6 | 28.6 | 28.6 | 14.3 | 85.7 | 0.0 | 0.0 | 14.3 |         |         |         |         |         |     |
| distant metastasis   | 0 | 25 | 12.0 | 0.0960 | 12.0 | 0.0960 | 8.0 | 0.0517 | 0.0 | 0.0404 | 84.0 | 8.0 | 0.0 | 8.0 | 0.5054 |
| ≥1                   | 4 | 50.0 | 50.0 | 50.0 | 25.0 | 75.0 | 0.0 | 0.0 | 25.0 |         |         |         |         |         |     |
Supplementary Figure 1: Multiplex fluorescence IHC of the used PD-L1 antibodies MSVA-011 (green) and E1L3N (red) revealed a high degree of co-expression (orange) (A and B). The PD-L1 expression and the density of PD-L1 positive cells was highly concordant across 28 cancer microenvironments (C).
Supplementary Figure 2. PD-L1 expression in cancer cells and immune cells and patient prognosis in papillary RCCs

- **Recurrence-free survival**
  - PD-L1 in cancer cells (cut-off ≥5%)
  - PD-L1 in cancer cells (cut-off ≥10%)
  - PD-L1 in cancer cells (cut-off ≥50%)
  - PD-L1 in immune cells (cut-off ≥50%)

- **Overall survival**
  - PD-L1 in cancer cells (cut-off ≥5%)
  - PD-L1 in cancer cells (cut-off ≥10%)
  - PD-L1 in cancer cells (cut-off ≥50%)
  - PD-L1 in immune cells

- **Tumor-specific death**
  - PD-L1 in cancer cells (cut-off ≥5%)
  - PD-L1 in cancer cells (cut-off ≥10%)
  - PD-L1 in cancer cells (cut-off ≥50%)
  - PD-L1 in immune cells