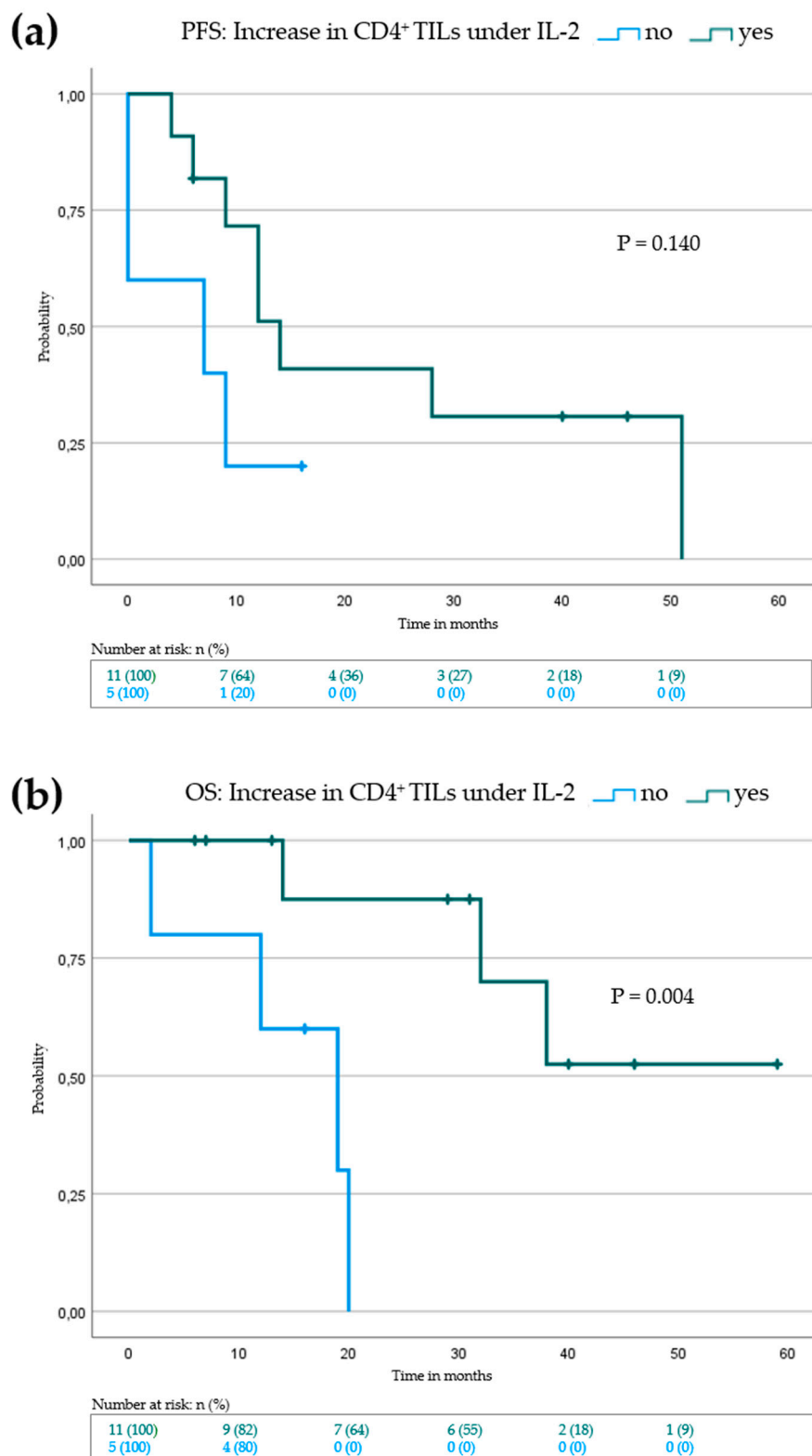
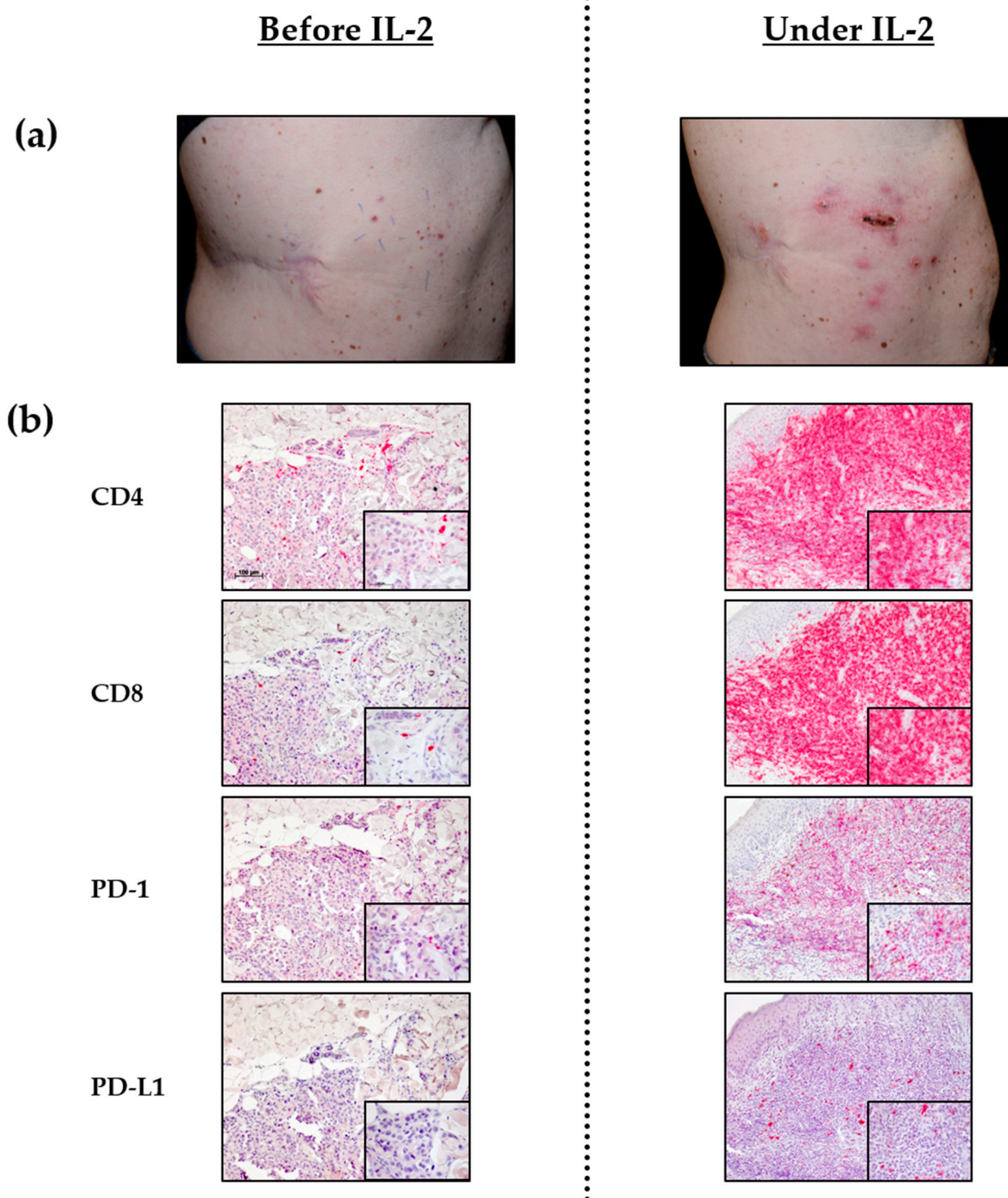


## Supplementary Figures



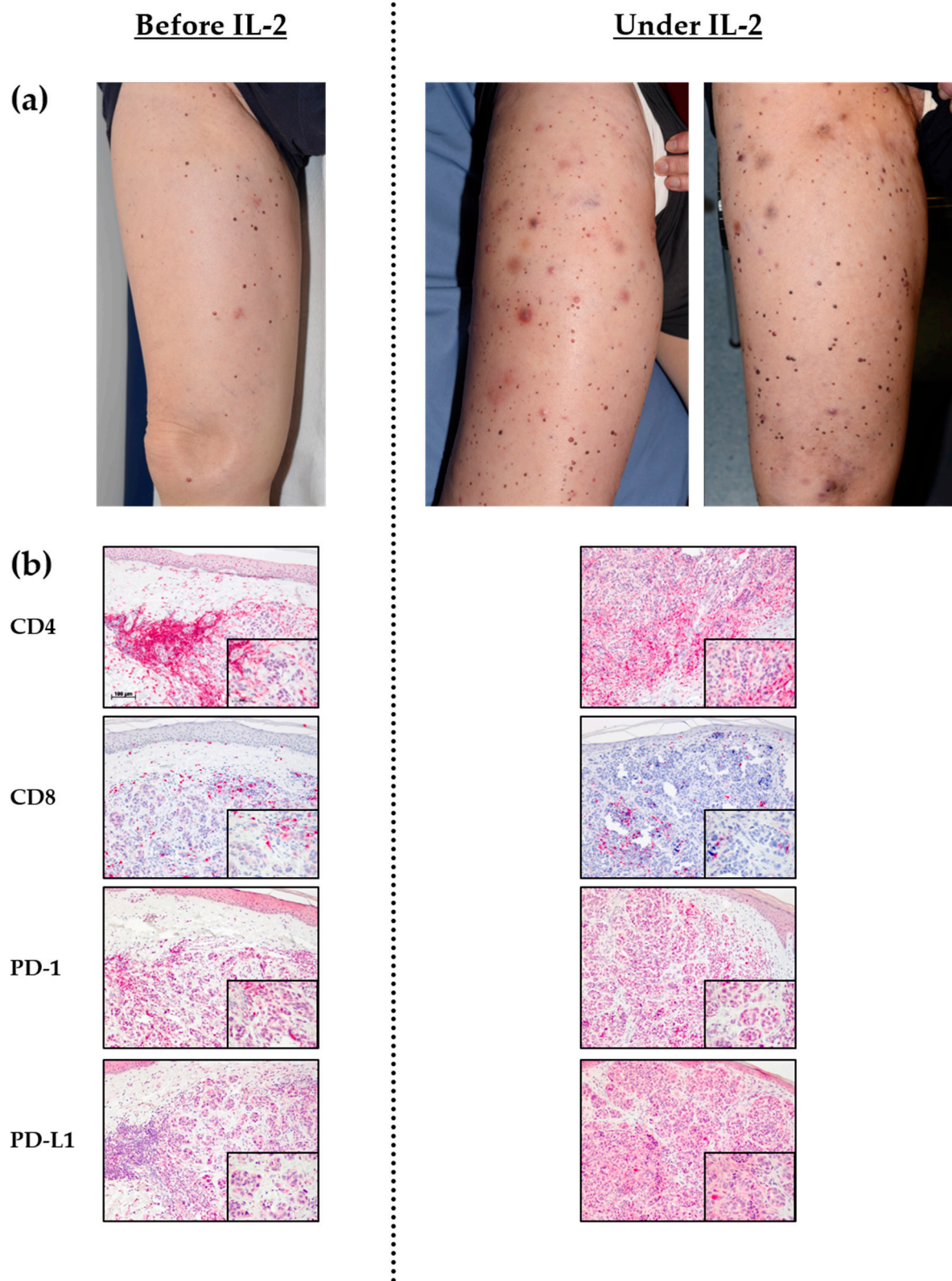
**Figure S1:** Kaplan-Meier survival blot showing progression free survival (PFS) in **(a)** and overall survival (OS) in **(b)** for patients with increase of CD4<sup>+</sup> tumor infiltrating lymphocytes (green curve) vs patients with no increase of CD4<sup>+</sup> tumor infiltrating lymphocytes (blue curve). Vertical dashes within the curves indicate censored patients. *P*-value indicates statistical significance of the log rank test.

## Patient #27 (loco regional CR)



**Figure S2:** Clinical course of patient 27: **(a)** Representative clinical pictures of cutaneous metastasis of the right flank before (left picture) and under IL-2 treatment (right picture: after 3 injections received, 18 MIU in total). **(b)** Representative immunohistochemistry staining of melanoma metastases. Biopsies were taken before and under treatment with IL-2. Scale bar = 100  $\mu$ m; Insert: scale bar = 50  $\mu$ m. CR = complete response.

### Patient #3 (loco regional and distant PD)



**Figure S3:** Clinical course of patient 3: (a) Representative clinical pictures of cutaneous metastasis of the right upper leg at different time points. i.e. before (left picture) and under IL-2 treatment (right picture: after 9 injections received, 66 MIU in total). (b) Representative immunohistochemistry staining of melanoma metastases. Biopsies were taken before and under treatment with IL-2. Scale bar = 100  $\mu$ m; Insert: scale bar = 50  $\mu$ m. PD = progressive disease.

**Supplementary Table S1:** Patient characteristics.

| Patient No | Age (Years) | Melanoma Type     | ECOG <sup>1</sup> | Stage at Beginning of IL-2 (AJCC <sup>2</sup> 2017) | Biomarkers before Start IL-2 |                  |                  | Increase of AEC <sup>5</sup> under IL-2 | Number of IL-2 Injections | Total Amount of IL-2 Applied (MIU <sup>6</sup> ) | Type of Injected Metastases      | IL-2-Related Adverse Events                      | Time Point of Follow up Biopsy Under IL-2 (Months) |
|------------|-------------|-------------------|-------------------|---|------------------------------|------------------|------------------|---|---------------------------|--|----------------------------------|--|--|
|            |             |                   |                   |   | LDH > ULN <sup>3</sup>       | S100 > ULN       | NLR <sup>4</sup> |   |                           |  |                                  |  |  |
| 1          | 69          | NM <sup>7</sup>   | 0                 | VI (pT3a pN1c M1c)                                  | no                           | no               | 7.39             | yes                                     | 8                         | 24   | C <sup>8</sup> , SC <sup>9</sup> | Pyrexia, Myalgia                                 | 7.0  |
| 2          | 69          | NM                | 0                 | IIIC (pT4a pN3c cM0)                                | yes                          | no               | 7.39             | yes                                     | 6                         | 18   | C                                | None   | 4.5  |
| 3          | 74          | ALM <sup>10</sup> | 1                 | IIIC (pT3b pN3c M0)                                 | no                           | no               | 0.22             | no                                      | 15                        | 124  | C, SC                            | Pyrexia, chills, eczema                          | 4.9  |
| 4          | 59          | ALM               | 0                 | IV (pT3b N1c M1a)                                   | no                           | no               | 1.02             | yes                                     | 19                        | 57   | C, SC, N <sup>11</sup>           | Dysesthesia, polyneuropathy, cramps, neutropenia | 8.2  |
| 5          | 65          | ALM               | 0                 | IV (pT4b pN3c M1d)                                  | no                           | no               | 1.56             | no                                      | 6                         | 19.5   | SC(d) <sup>12</sup>              | None   | 2.6  |
| 6          | 52          | mucosal           | 1                 | IV (pT4b pN2c M1c)                                  | yes                          | yes              | 1.68             | no                                      | 12                        | 42   | C                                | Nausea, impaired vision, local inflammation      | 2.4  |
| 7          | 61          | NM                | 0                 | IIIB (pT2a pN1c M0)                                 | no                           | yes              | 1.99             | yes                                     | 28                        | 252  | SC, N                            | Asthmatic symptoms, pyrexia                      | 1.1  |
| 8          | 66          | NM                | 0                 | IV (pT3b pN3c M1c)                                  | no                           | yes              | 3.76             | no                                      | 3                         | 21   | C, SC                            | Pyrexia, chills, vomiting, diarrhea              | 0.5  |
| 9          | 85          | SSM <sup>13</sup> | 0                 | IV (pT4b pN1c cM1b)                                 | no                           | no               | 2.46             | yes                                     | 13                        | 102  | SC                               | Pyrexia  | 1.3  |
| 10         | 54          | SSM               | 0                 | IV (pT2b pN3c cM1d)                                 | yes                          | NA <sup>14</sup> | 2.62             | no                                      | 2                         | 9  | C                                | none   | NA   |
| 11         | 36          | ALM               | 0                 | IIIC (pT3b pN2c cM0)                                | no                           | no               | 1.90             | yes                                     | 13                        | 58.5   | C                                | Local edema                                      | 1.3  |
| 12         | 81          | ALM               | 0                 | IIIC (pT4b pN2c cM0)                                | no                           | no               | 1.44             | no                                      | 5                         | 16.5   | C                                | Local inflammation                               | NA   |
| 13         | 80          | NM                | 0                 | IIIC (pT4b pN1c cM0)                                | yes                          | no               | 5.02             | yes                                     | 4                         | 6.6  | C                                | None   | NA   |
| 14         | 88          | SSM               | 0                 | IIIC (pT4b pN2c cM0)                                | yes                          | no               | 2.30             | yes                                     | 12                        | 102  | C, SC                            | Pyrexia  | NA   |
| 15         | 84          | NM                | 1                 | IV (pT3b pN3c M1a)                                  | NA <sup>3</sup>              | NA               | 3.77             | yes                                     | 2                         | 12   | C, SC                            | Local inflammation                               | NA   |
| 16         | 28          | CUP <sup>15</sup> | 0                 | IV (T0 Nx M1a)                                      | no                           | no               | 1.75             | yes                                     | 21                        | 96   | SC, N                            | Leucopenia, fatigue, local inflammation          | 1.7  |
| 17         | 82          | Uveal             | 1                 | IV (Tx cN0 cM1c)                                    | yes                          | no               | NA               | NA                                      | 8                         | 144  | C(d) <sup>16</sup> , SC(d)       | Pyrexia  | NA   |
| 18         | 69          | ALM               | 0                 | IV (pT4b pN1c cM1b)                                 | yes                          | no               | 6.37             | yes                                     | 14                        | 180  | C, SC, N                         | Pyrexia, Erysipelas                              | 1.5  |
| 19         | 73          | DM <sup>17</sup>  | 1                 | IV (pT3b pN3c cM1c)                                 | yes                          | NA               | NA               | no                                      | 5                         | 99   | C, SC, N                         | None   | NA   |
| 20         | 73          | ALM               | 0                 | IV (pT4b pN2c cM1b)                                 | no                           | no               | NA               | yes                                     | 41                        | 300  | C, N                             | Pyrexia  | 13.4   |
| 21         | 75          | ALM               | 0                 | IIIC (pT4 pN3c cM0)                                 | NA                           | NA               | 2.92             | no                                      | 25                        | 186  | C, SC                            | Pyrexia  | NA   |
| 22         | 68          | NM                | 0                 | IIIC (pT3b pN2c cM0)                                | NA                           | NA               | 3.66             | yes                                     | 17                        | 150  | C                                | Fatigue, dysesthesia, local inflammation         | 10.1   |
| 23         | 71          | ALM               | 0                 | IV (pT4b pN3b cM1c)                                 | NA                           | NA               | 3.10             | no                                      | 14                        | 84   | C(d)                             | Local itching, flush                             | NA   |
| 24         | 83          | NM                | 0                 | IIIB (pT3b pN1c cM0)                                | NA                           | NA               | 2.22             | no                                      | 5                         | 30   | C, SC, N                         | None   | 4.1  |
| 25         | 77          | ALM               | 0                 | IV (pT4b pN3c cM1c)                                 | no                           | yes              | 2.58             | no                                      | 7                         | 27   | C                                | 2 x Syncope                                      | NA   |
| 26         | 83          | ALM               | 1                 | IV (pT3b cN2c cM1b)                                 | no                           | yes              | 2.37             | no                                      | 9                         | 93   | C, SC                            | Local pain                                       | NA   |
| 27         | 76          | SSM               | 0                 | IIIC (pT3b pN3c cM0)                                | NA                           | NA               | 3.03             | NA                                      | 9                         | 40.5   | C                                | Pyrexia, local inflammation, dyspnea, diarrhea   | 5.1  |

---

<sup>1</sup>Eastern Cooperative Oncology Group performance status score. <sup>2</sup>American Joint Committee on Cancer, Melanoma of the Skin Staging, 8th edition. <sup>3</sup>Upper limit normal. <sup>4</sup>Neutrophil-to-lymphocyte ratio. <sup>5</sup> Absolute eosinophil count. <sup>6</sup> Million International Units. <sup>7</sup> Nodular melanoma. <sup>8</sup> Cutaneous (locoregional) <sup>9</sup> Subcutaneous (locoregional). <sup>10</sup>Acral lentiginous melanoma. <sup>11</sup> Nodal (locoregional) <sup>12</sup> Subcutaneous (distant). <sup>13</sup> Superficial spreading melanoma <sup>14</sup> Not available. <sup>15</sup> Cancer of unknown primary. <sup>16</sup> Cutaneous (distant) <sup>17</sup> Desmoplastic melanoma.

**Supplementary Table S2.** Overall survival times (baseline characteristics).

| Condition                                    | Median OS | 95% CI <sup>1</sup> | <i>p</i> Values |
|--|-----------|---------------------|-----------------|
| <b>Sex</b>                                   |           |                     |                 |
| Female ( <i>n</i> = 12)                      | 32        | 0.0–65.0            | 0.384           |
| Male ( <i>n</i> = 15)                        | 20        | 12.9–27.1           |                 |
| <b>Age over 70</b>                           |           |                     |                 |
| No ( <i>n</i> = 12)                          | 38        | 0.0–81.7            | 0.238           |
| Yes ( <i>n</i> = 15)                         | 20        | 5.2–34.8            |                 |
| <b>Loco regional Progression on</b>          |           |                     |                 |
| Adjuvant therapy ( <i>n</i> = 9)             | 32        | -*                  | 0.090           |
| Palliative therapy ( <i>n</i> = 18)          | 14        | 5.2–22.8            |                 |
| <b>Active distant metastases at baseline</b> |           |                     |                 |
| No ( <i>n</i> = 13)                          | NR        | -                   | <0.001          |
| Yes ( <i>n</i> = 14)                         | 12        | 2.8–21.2            |                 |
| <b>Baseline LDH<sup>2</sup></b>              |           |                     |                 |
| Normal ( <i>n</i> = 13)                      | 32        | 4.9–59.2            | 0.171           |
| Elevated ( <i>n</i> = 8)                     | 14        | 5.7–22.3            |                 |
| <b>Baseline S100</b>                         |           |                     |                 |
| Normal ( <i>n</i> = 14)                      | 32        | 13.0–51.0           | 0.325           |
| Elevated ( <i>n</i> = 5)                     | 14        | 0.0–31.2            |                 |
| <b>Baseline NLR<sup>3</sup></b>              |           |                     |                 |
| ≤2.5 ( <i>n</i> = 12)                        | 32        | 8.9–55.1            | 0.533           |
| >2.5 ( <i>n</i> = 12)                        | 19        | 0.0–41.0            |                 |
| <b>BRAF-V600-Mutation</b>                    |           |                     |                 |
| Wild type ( <i>n</i> = 22)                   | 20        | 18.1–21.9           | 0.216           |
| Positive (5)                                 | NR        | -                   |                 |

<sup>1</sup> 95% confidence interval of median. <sup>2</sup> Lactate dehydrogenase. <sup>3</sup> Neutrophil-to-lymphocyte ratio.

\* No CI due to low event numbers. NR = median not reached. *P*-value indicates statistical significance of the log rank test.

**Supplementary Table S3.** Overall survival times (values under therapy)

| Condition   | Median OS | 95% CI <sup>1</sup> | <i>p</i> Values |
|---|-----------|---------------------|-----------------|
| <b>Increase of AEC<sup>2</sup> under IL-2</b>       |           |                     |                 |
| No ( <i>n</i> = 12)                                 | 13        | 5.9–20.0            | <0.001          |
| Yes ( <i>n</i> = 13)                                | 38        | -*                  |                 |
| <b>Loco regional response to IL-2</b>               |           |                     |                 |
| No ( <i>n</i> = 8)                                  | 8         | 0.0–17.7            | <0.001          |
| Yes ( <i>n</i> = 19)                                | 38        | 23.9–52.1           |                 |
| <b>Increase in CD4<sup>+</sup> TILs<sup>3</sup></b> |           |                     |                 |
| No ( <i>n</i> = 5)                                  | 19        | 8.1–29.9            | 0.004           |
| Yes ( <i>n</i> = 11)                                | NR        | -                   |                 |
| <b>Increase in CD8<sup>+</sup> TILs</b>             |           |                     |                 |
| No ( <i>n</i> = 4)                                  | 12        | 0.0–28.7            | <0.001          |
| Yes ( <i>n</i> = 12)                                | NR        | -                   |                 |
| <b>Increase in PD-1 staining<sup>4</sup></b>        |           |                     |                 |
| No ( <i>n</i> = 6)                                  | 19        | 9.4–28.6            | 0.194           |
| Yes ( <i>n</i> = 10)                                | 38        | 25.7–50.3           |                 |
| <b>Increase in PD-L1 staining<sup>5</sup></b>       |           |                     |                 |
| No ( <i>n</i> = 9)                                  | 38        | 15.4–60.6           | 0.667           |
| Yes ( <i>n</i> = 7)                                 | 32        | 5.1–58.9            |                 |

<sup>1</sup> 95% confidence interval of median <sup>2</sup> Absolute eosinophil count. <sup>3</sup> Tumor infiltrating lymphocytes.  
<sup>4</sup> Programmed Cell Death Protein 1. <sup>5</sup> Programmed Cell Death 1 Ligand 1. \* No CI due to low event numbers. NR = median not reached. *P*-value indicates statistical significance of the log rank test.

**Supplementary Table S4.** Progression free survival times (Cancer of unknown primary, uveal and mucosal melanoma excluded).

| Condition   | Median PFS | 95% CI <sup>1</sup> | <i>p</i> Values |
|---|------------|---------------------|-----------------|
| <b>Active distant metastases at baseline</b>        |            |                     |                 |
| No ( <i>n</i> = 13)                                 | 14         | 0.0–33.5            | 0.005           |
| Yes ( <i>n</i> = 11)                                | 3          | 0.0–7.0             |                 |
| <b>Increase of AEC<sup>2</sup> under IL-2</b>       |            |                     |                 |
| No ( <i>n</i> = 11)                                 | 3          | 0.0–6.2             | <0.001          |
| Yes ( <i>n</i> = 12)                                | 14         | 0.0–32.9            |                 |
| <b>Loco regional response to IL-2</b>               |            |                     |                 |
| No ( <i>n</i> = 6)                                  | 0          | -*                  | <0.001          |
| Yes ( <i>n</i> = 18)                                | 12         | 4.5–19.5            |                 |
| <b>Increase in CD4<sup>+</sup> TILs<sup>3</sup></b> |            |                     |                 |
| No ( <i>n</i> = 5)                                  | 7          | 0.0–22.0            | 0.224           |
| Yes ( <i>n</i> = 9)                                 | 14         | 7.5–20.5            |                 |
| <b>Increase in CD8<sup>+</sup> TILs</b>             |            |                     |                 |
| No ( <i>n</i> = 4)                                  | 0          | -*                  | 0.010           |
| Yes ( <i>n</i> = 10)                                | 14         | 0.0–36.2            |                 |
| <b>Increase in PD-1 staining<sup>4</sup></b>        |            |                     |                 |
| No ( <i>n</i> = 5)                                  | 7          | 0.0–22.0            | 0.224           |
| Yes ( <i>n</i> = 9)                                 | 14         | 7.5–20.5            |                 |
| <b>Increase in PD-L1 staining<sup>5</sup></b>       |            |                     |                 |
| No ( <i>n</i> = 8)                                  | 9          | 2.1–15.9            | 0.795           |
| Yes ( <i>n</i> = 6)                                 | 9          | 3.1–14.9            |                 |

<sup>1</sup> 95% confidence interval of median. <sup>2</sup> Absolute eosinophil count. <sup>3</sup> Tumor infiltrating lymphocytes.

<sup>4</sup> Programmed Cell Death Protein 1. <sup>5</sup> Programmed Cell Death 1 Ligand 1. \* No CI due to low event numbers. *P*-value indicates statistical significance of the log rank test.



**Supplementary Table S5.** Overall survival times (Cancer of unknown primary, uveal and mucosal melanoma excluded).

| Condition   | Median OS | 95% CI <sup>1</sup> | <i>p</i> Values |
|---|-----------|---------------------|-----------------|
| <b>Active distant metastases at baseline</b>        |           |                     |                 |
| No ( <i>n</i> = 13)                                 | NR        | -                   | <0.001          |
| Yes ( <i>n</i> = 11)                                | 12        | 6.3–17.7            |                 |
| <b>Increase of AEC<sup>2</sup> under IL-2</b>       |           |                     |                 |
| No ( <i>n</i> = 11)                                 | 12        | 5.6–18.4            | <0.001          |
| Yes ( <i>n</i> = 12)                                | 38        | -*                  |                 |
| <b>Loco regional response to IL-2</b>               |           |                     |                 |
| No ( <i>n</i> = 6)                                  | 8         | 0.0–20.0            | <0.001          |
| Yes ( <i>n</i> = 18)                                | 38        | 23.6–52.4           |                 |
| <b>Increase in CD4<sup>+</sup> TILs<sup>3</sup></b> |           |                     |                 |
| No ( <i>n</i> = 5)                                  | 19        | 8.1–29.9            | 0.001           |
| Yes ( <i>n</i> = 9)                                 | NR        | -                   |                 |
| <b>Increase in CD8<sup>+</sup> TILs</b>             |           |                     |                 |
| No ( <i>n</i> = 4)                                  | 12        | 0.0–28.7            | <0.001          |
| Yes ( <i>n</i> = 10)                                | NR        | -                   |                 |
| <b>Increase in PD-1 staining<sup>4</sup></b>        |           |                     |                 |
| No ( <i>n</i> = 5)                                  | 19        | 3.9–34.0            | 0.052           |
| Yes ( <i>n</i> = 9)                                 | 38        | -*                  |                 |
| <b>Increase in PD-L1 staining<sup>5</sup></b>       |           |                     |                 |
| No ( <i>n</i> = 8)                                  | 20        | 17.5–22.5           | 0.289           |
| Yes ( <i>n</i> = 6)                                 | 32        | -*                  |                 |

<sup>1</sup> 95% confidence interval of median <sup>2</sup> Absolute eosinophil count. <sup>3</sup> Tumor infiltrating lymphocytes.

<sup>4</sup> Programmed Cell Death Protein 1. <sup>5</sup> Programmed Cell Death 1 Ligand 1. \* No CI due to low event numbers. NR = median not reached. *P*-value indicates statistical significance of the log rank test.