

SUPPLEMENTARY TABLES

Supplementary table 1: Antibody panels for phenotype and reactivity testing.

Antibodies used for phenotype and anti-tumor reactivity testing. *used for intracellular staining.

| Panel | Antigen | Flouochrome | Clone | Company |
|-------------|--------------|--------------|------------|------------|
| Pheno1 | CD57 | FITC | NK-1 | BD |
| Pheno1 | CD28 | APC | CD28.2 | BD |
| Pheno1 | CD27 | BV711 | L123 | BD |
| Pheno1 | CCR7 | BV421 | G043H7 | Biolegend |
| Pheno1 | CD45RA | BV650 | HI100 | BD |
| Pheno1+2 | CD103 | PE | Ber-ACT8 | BD |
| Pheno1+2 | CD39 | PE-Cy7 | eBioA1(A1) | Invitrogen |
| Pheno1+2 | CD56 | BV605 | NCAM16.2 | BD |
| Pheno1+2 | CD8 | APC-R700 | RPA-T8 | BD |
| Pheno1+2 | CD4 | BV510 | SK3 | BD |
| Pheno1+2 | CD3 | BV786 | OKT3 | BD |
| Pheno1+2 | PD-1 | PE-Dazzle594 | EH12.2H7 | Biolegend |
| Pheno2 | LAG3 | FITC | 11C3C65 | Biolegend |
| Pheno2 | CD137 | APC | 4B4-1 | Biolegend |
| Pheno2 | BTLA | BV421 | J168-540 | BD |
| Pheno2 | TIGIT | BV650 | 741182 | BD |
| Pheno2 | TIM3 | BV711 | 7D3 | BD |
| Reactivity* | CD137 | PE | 4B4-1 | BD |
| Reactivity | CD56 | BV510 | NCAM16.2 | BD |
| Reactivity | CD4 | BV711 | SK3 | BD |
| Reactivity | CD3 | PE-CF594 | UCHT1 | BD |
| Reactivity | CD8 | Qdot 605 | 3B5 | Invitrogen |
| Reactivity* | IFN γ | PE-Cy7 | B27 | BD |
| Reactivity* | TNF | APC | MAb11 | BD |
| Reactivity | CD56 | PE | NCAM16.2 | BD |

Supplementary table 2: Overview of all successful *in vitro* TIL expansions and clinical efficacy. Patient and resected tumor characteristics of patients where a young TIL product could be established. Duration of the initial *in vitro* expansion (Exp time) is shown in days. For patients were treated with the REP TILs, the fold expansion (Fold exp) during the rapid expansion protocol (REP), the total number of REP TILs, and the clinical response to therapy is also listed. TMB: Tumor mutational burden; BOR: Best overall response; PRS: Progression-free survival.

| ID | Diagnosis | Metastasis | Exp. time | Fold exp. | REP TILs (10e9) | CD4/CD8 (%) | TMB (Mut./Mb) | BOR | PFS (days) | OS (days) |
|--------|------------------------------|-------------------|-----------|-----------|-----------------|-------------|---------------|--------|------------|-----------|
| 1 | Colon cancer | Peritoneum | 44 | 265 | 5.3 | 92.3/5.4 | 7.1 | SD | 96 | 119 |
| 2 | Colon cancer | Lung | 18 | 6300 | 126.0 | 48.8/45.5 | 4.7 | SD | 31 | 63 |
| 3 | Colon cancer | Lung | 18 | 2780 | 55.6 | 74.8/26.3 | 11 | SD | 190 | 227 |
| 4 | Colon cancer | Lung | 19 | 3460 | 69.2 | 59.4/32.6 | 7.1 | SD | 48 | 110 |
| 5 | Colon cancer | Pleura | 27 | 1645 | 32.9 | 95.8/1.4 | 7.1 | PD | 50 | 83 |
| 6 | Rectal cancer | Lung | 20 | 4380 | 87.6 | 4.8/76.8 | 7.2 | SD | 168 | 517 |
| 7 | Rectal cancer | Lung | 26 | 3620 | 72.4 | 89.4/5.9 | 10.2 | SD | 201 | 787 |
| 8 | Cholangiocarcinoma | Lung | 15 | 4520 | 90.4 | 87.0/11.1 | 3.1 | PR | 199 | 870 |
| 9 | Cholangiocarcinoma | Lung | 20 | 4420 | 88.4 | 92.0/6.1 | 1.6 | SD | 26 | 50 |
| 10 | Cholangiocarcinoma | Subcutis (thorax) | 23 | 2840 | 56.8 | 25.3/61.6 | 3.9 | SD | 134 | 512 |
| 11 | Melanoma (skin) | Adrenal gland | 29 | 2550 | 51.0 | 96.4/2.5 | 5.5 | PD | 47 | 179 |
| 12 | Melanoma (skin) | Subcutis (thorax) | 20 | 2610 | 78.3 | 20.2/65.5 | 9.4 | PD | 12 | 77 |
| 13 | Melanoma (choridea) | Lung | 28 | 3060 | 61.2 | 40.5/54.2 | 2.4 | SD | 172 | 412 |
| 14 | Melanoma (choridea) | Pleura | 36 | 3280 | 65.6 | 2.0/4.1 | 1.6 | SD | 44 | 772 |
| 15 | Melanoma (choridea) | Liver | 26 | 2660 | 53.2 | 82.4/25.2 | n/a | SD | 211 | 393 |
| 16 | Melanoma (choridea) | Liver | 19 | 3860 | 77.2 | 59.4/40.8 | 0.8 | SD | 84 | 142 |
| 17 | Leiomyosarcoma | Lung | 34 | 1740 | 34.8 | 45.7/43.8 | 2.4 | SD/uPR | 87 | 251 |
| 18 | Leiomyosarcoma | Subcutis (thorax) | 36 | 2320 | 46.4 | 62.3/20.2 | 0.8 | SD | 86 | 540 |
| 19 | Oropharyngeal cancer (HNSCC) | Lung | 19 | 4748 | 109.2 | 22.9/65.6 | 8.6 | PR | 90 | 370 |
| 20 | Cervix cancer | Lung | 19 | 4960 | 99.2 | 81.8/22.7 | 11.8 | SD | 89 | 218 |
| 21 | Myoepithelial carcinoma | LN (neck) | 29 | 2568 | 48.8 | 82.5/22.9 | 0.8 | SD | 93 | 521 |
| 22 | Adrenocorticocarcinoma | LN (neck) | 23 | 3170 | 63.4 | 45.4/48.1 | 16.5 | SD | 104 | 122 |
| 23 | Non-small cell lung cancer | Lung | 25 | 2600 | 52.0 | 53.6/28.8 | 0.8 | SD | 85 | 159 |
| 24 | Rhinopharyngeal cancer | Lung | 19 | 2760 | 55.2 | 49.2/36.1 | 8.7 | SD/uPR | 155 | 448 |
| 25 | Thyroid cancer | Liver | 27 | 1990 | 39.8 | 64.8/48.6 | 5.50 | PD | 51 | 71 |
| 26 | Melanoma (mucosal) | Lung | 19 | - | - | | | | | |
| 27 | Colon cancer | Liver | 25 | - | - | | | | | |
| 28 | Breast cancer | Skin | 24 | - | - | | | | | |
| 29 | Breast cancer | Skin (thorax) | 23 | - | - | | | | | |
| 30 | Pancreatic cancer | Liver | 31 | - | - | | | | | |
| Median | | | 24 | 2840 | 61.2 | | | | 89 | 227 |

SUPPLEMENTARY FIGURES

Supplementary Figure 1: *In vitro* anti-tumor reactivity of all treated patients.

The expanded TILs (REP TILs) were tested for anti-tumor reactivity with flow cytometry after co-culture with unstimulated autologous tumor cells. REP TILs with expression of at least two markers (TNF α , IFN γ , CD107a or CD137) in than 0.5% of all CD3 $^{+}$ cells were considered anti-tumor reactive (indicated by dotted line). Five patients had high background reactivity of REP TILs (#14) or the tumor derived material (#4, #15, #16, #23) so that anti-tumor specific reactivity of the REP TILs could not be determined. ACC: Adenocorticocarcinoma; CRC: Colorectal cancer; CC: Cholangiocarcinoma; HNSCC: Head-and-neck squamous cell carcinoma; MM: Malignant Melanoma; MEC: Myoepithelial carcinoma; NSCLC: Non-small cell lung cancer. *Patients in the disease control group.

