### Supplementary Table S1. Demographic and baseline characteristics

| Characteristic                              | NLR<sup>low</sup> (n = 10) | NLR<sup>high</sup> (n = 12) | P value |
|---------------------------------------------|-----------------------------|-----------------------------|---------|
| Age at diagnosis - yr                       |                             |                             |         |
| Median                                      | 58                          | 59                          | 0.55    |
| Range                                       | 40-72                       | 51-81                       | -       |
| Sex - no. (%)                               |                             |                             |         |
| Male                                        | 8 (80)                      | 6 (50)                      | 0.2     |
| Female                                      | 2 (20)                      | 6 (50)                      | 0.2     |
| Race or ethnic group - no. (%)              |                             |                             |         |
| White                                       | 8 (80)                      | 11 (92)                     | 0.57    |
| Black                                       | 1 (10)                      | 1 (8)                       | >0.9    |
| Asian                                       | 1 (10)                      | 0 (0)                       | 0.45    |
| ECOG - no. (%)                              |                             |                             |         |
| 0                                           | 5 (50)                      | 4 (33)                      | 0.67    |
| 1                                           | 5 (50)                      | 8 (66)                      | 0.67    |
| Extent of Disease - no. (%)                 |                             |                             |         |
| Locally Advanced                            | 2 (20)                      | 0 (0)                       | 0.19    |
| Metastatic                                  | 8 (80)                      | 12 (100)                    | 0.19    |
| Location of Primary - no (%)                |                             |                             |         |
| Head                                        | 2 (20)                      | 6 (50)                      | 0.2     |
| Body or Tail                                | 7 (70)                      | 4 (33)                      | 0.2     |
| Unknown                                     | 1 (10)                      | 2 (16)                      | >0.9    |
| Site of Metastases - no. (%)                |                             |                             |         |
| Liver                                       | 7 (70)                      | 12 (100)                    | 0.078   |
| Lung                                        | 0 (0)                       | 1 (8)                       | >0.9    |
| Peritoneal                                  | 3 (30)                      | 0 (0)                       | 0.078   |
| Bone                                        | 0 (0)                       | 2 (17)                      | 0.48    |
| Other                                       | 1 (10)                      | 1 (8)                       | >0.9    |
| Prior Radiation Therapies - no. (%)         |                             |                             |         |
| No                                          | 10 (100)                    | 11 (92)                     | >0.9    |
| Yes                                         | 0 (0)                       | 1 (8)                       | >0.9    |

### Supplementary Table S2. Survival comparison

| Study                           | Treatment                  | n   | NLR cutoff | median OS |
|---------------------------------|----------------------------|-----|------------|-----------|
| Wattenberg et al                | CD40 agonist and gemcitabine | 10  | < 5        | 11.7      |
|                                 |                            | 12  | > 5        | 5.8       |
| Goldstein et al                 | Gemcitabine plus nab-paclitaxel | 266 | < 5        | 10.9      |
|                                 | Gemcitabine                | 160 | > 5        | 5.6       |
|                                 |                            | 277 | < 5        | 7.9       |
|                                 |                            | 149 | > 5        | 4.3       |
| Antibody           | Source  | Product # | Tag     | Clone  |
|--------------------|---------|-----------|---------|--------|
| CD196/CCR6         | Fluidigm| 3141014A  | 141Pr   | 11A9   |
| CD11a              | Fluidigm| 3142006B  | 142Nd   | HI111  |
| CD123              | Fluidigm| 3143014B  | 143Nd   | 6H6    |
| CD38               | Fluidigm| 3144014B  | 144Nd   | HIT2   |
| CD4                | Fluidigm| 3145001B  | 145Nd   | RPAT4  |
| CD64               | Fluidigm| 3146006B  | 146Nd   | 10.1   |
| CD11c              | Fluidigm| 3147008B  | 147Sm   | Bu15   |
| CD16               | Fluidigm| 314800rB  | 148Nd   | WM53   |
| CD66a              | Fluidigm| 3149018B  | 149Sm   | ASL32  |
| MIP1beta           | Fluidigm| 3150004B  | 150Nd   | D211351|
| LAMP1              | Fluidigm| 3151002B  | 151Eu   | H4A3   |
| TNFa               | Fluidigm| 3152002B  | 152Sm   | Mab11  |
| BDCA-2/CD303       | Fluidigm| 3153007B  | 153Eu   | 201A   |
| CD163              | Fluidigm| 3154007B  | 154Sm   | GHI/61 |
| CD1b               | Fluidigm| 3155007B  | 155Gd   | SN13   |
| CD86               | Fluidigm| 3156008B  | 156Gd   | IT2.2  |
| CD169              | Fluidigm| 3158027B  | 158Gd   | CD169  |
| PD-L1              | Fluidigm| 3159029B  | 159Tb   | 29E.2A3|
| CD14               | Fluidigm| 3160001B  | 160Gd   | M5E2   |
| CD80               | Fluidigm| 3161023B  | 161Dy   | 2D10.4 |
| CD8a               | Fluidigm| 3162015B  | 162Dy   | RPAT8  |
| CD33               | Fluidigm| 3163023B  | 163Dy   | WM53   |
| CD15               | Fluidigm| 3164001B  | 164Dy   | W6D3   |
| CD40               | Fluidigm| 3165005B  | 165Ho   | 5C3    |
| CD34               | Fluidigm| 3166012B  | 166Er   | 581    |
| CD1a               | Fluidigm| 3167012B  | 167Er   | HI149  |
| CD206              | Fluidigm| 3168008B  | 168Er   | 152    |
| CD19               | Fluidigm| 3169011B  | 169Tm   | HIB19  |
| CD3                | Fluidigm| 3170001B  | 170Er   | UCHT1  |
| CXCR5              | Fluidigm| 3171014B  | 171Yb   | RF8B2  |
| CX3CR1             | Fluidigm| 3172017B  | 172Yb   | 2A91   |
| CD141              | Fluidigm| 3173002B  | 173Yb   | 1A4    |
| HLA-DR             | Fluidigm| 3174001B  | 174Yb   | L243   |
| PD1                | Fluidigm| 3175008B  | 175Lu   | EH12.2H7|
| CD56               | Fluidigm| 3176003B  | 176Yb   | CMSSB  |
| CD11b              | Fluidigm| 3209003B  | 209Bi   | ICRF44 |
| CD45               | Fluidigm| 3089003B  | Y89     | H130   |
Supplementary Figure S1.

(A) Study design. Black boxes indicate blood collection. G, gemcitabine; C, CP-870-893. (B) Heatmap showing relative mean marker expression values of CD45+ FlowSOM defined clusters. Patient and healthy volunteer samples were concatenated prior to analysis. (C-E) Quantification of cluster frequency. Mean ± SEM is shown. Day 1, n = 17; day 3, n = 13; day 5, n = 12; day 8, n = 15; day 15, n = 7, cycle 2, n = 14, cycle 3, n = 11. Mixed effects analysis with Dunnett’s multiple comparison test was performed. *, p < 0.05; **, p < 0.01.
Supplementary Figure S2. Gating strategy
Supplementary Figure S3. | See next page for caption.
Supplementary Figure S3.

(A) Heatmap of plasma cytokines. Each column is an individual patient (n = 19). (B) Quantification of absolute eosinophil, basophil and platelet counts among HV (n = 4), NLR\textsubscript{low} (n = 10) and NLR\textsubscript{high} (n = 12) patients. (C) Quantification of absolute counts of immune cell populations among NLR\textsubscript{high} (n = 10) and NLR\textsubscript{low} (n = 7) patients. Absolute counts were calculated by multiplying the indicated cell subset (as a percentage of CD45\textsuperscript{+} cells) times the total white blood cell count minus granulocytes. (D) Quantification of manually gated immune cell populations (as percentage of CD45\textsuperscript{+} cells). (E) Quantification of CD4:CD8 T cell ratio. (F) Quantification of CD45\textsuperscript{+} FlowSOM defined cluster frequencies which are > 1% of CD45\textsuperscript{+} cells. Clusters are defined as in Supplementary Figure S1B. Mean ± SEM is shown. ANOVA with Tukey’s correction for multiple comparisons (B, E), Mann-whitney tests (C) and Multiple t-tests were performed with Benjamini and Hochberg correction (F) were performed. *, p < 0.05. HV, healthy volunteer.
Supplementary Figure S4.

(A) Purity of bead selected CD14\(^+\) cells by flow cytometry. (B) Differential gene expression among monocytes from patients with low plasma cytokines (pCytokine\(^{\text{low}}\); IL-6 < 10 pg/mL and IL-8 < 45 pg/mL) and high plasma cytokines (pCytokine\(^{\text{high}}\); IL-6 > 10 pg/mL and IL-8 > 45 pg/mL). Significance analysis of microarray was used with FDR < 0.2.
Supplementary Figure S5. | See next page for caption.
Supplementary Figure S5.

(A) Neutrophil-lymphocyte ratio (NLR) in the peripheral blood over one cycle of treatment with gemcitabine and anti-CD40 therapy (n = 22). Dashed line represents NLR cutoff. Patients stratified by baseline neutrophil-lymphocyte ratio (NLR) as NLR\text{low} (NLR < 3.1, blue) or NLR\text{high} (NLR > 3.1, red). Multiple t-tests were performed with Benjamini and Hochberg correction. (B-J) Quantification of cluster frequency. Clusters are defined as in Supplementary Figure S1B. Patients were defined by baseline NLR as NLR\text{low} (<3.1) or NLR\text{high} (>3.1). Mean ± SEM is shown. Day 1, n = 17; day 3, n = 13; day 5, n = 12; day 8, n = 15, day 15, n = 7, day 28, n = 14. Mixed effects analysis with Dunnett’s multiple comparison test was performed. *, p < 0.05.
### Supplementary Figure S6.

**A** Multivariate survival analysis using sex, age, ECOG performance status, tumor burden (sum of baseline RECIST measurements) and neutrophil-lymphocyte ratio greater or less than 3.1.

| Endpoint          | Subgroup     | n  | HR     | CI      | p     |
|-------------------|--------------|----|--------|---------|-------|
| OS                | Female       | 22 | 0.81   | 0.28 - 2.37 | 0.704 |
| ECOG performance status |             |    | 0.97   | 0.33 - 2.79 | 0.949 |
| Tumor burden (sum of target lesions) |             |    | 0.99   | 0.92 - 1.06 | 0.769 |
| Age               |              |    | 1.02   | 0.96 - 1.08 | 0.480 |
| Neutrophil lymphocyte ratio >3.1 |          |    | 3.87   | 1.04 - 14.38 | 0.043 |