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# Supplemental Figures



## Figure S1: Patient disposition in the dose-escalation portion of PIVOT-02

\*Of the three active patients, one discontinued BEMPEG but continued NIVO. †Of the six active patients, three discontinued BEMPEG but continued NIVO.

1L, first line; 2L, second line; BEMPEG, bempegaldesleukin; I-O, immunotherapy; NIVO, nivolumab; NSCLC, non-small cell lung carcinoma; PD, progressive disease; q2w, every 2 weeks; q3w, every 3 weeks; RCC, renal cell carcinoma; RECIST, Response Evaluation Criteria In Solid Tumors.

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## Figure S2: Cytokine-related TEAEs of interest by cycle (N=38)

\*Includes the following preferred terms: chills, influenza-like illness, pyrexia.

†Includes the following preferred terms: erythema, rash, rash erythematous, rash generalized, rash macular, rash maculo-papular, rash maculovesicular, rash papular, rash pruritic, rash pustular, rash vesicular, and exfoliative rash.

TEAE, treatment-emergent adverse event.



Figure S3: Swimmers plots by tumor type for the response-evaluable population (n=37). End of treatment is based on date and reason for discontinuing both BEMPEG and NIVO, whichever is later. Other reasons for end of treatment include adverse event, death, lost to follow-up, noncompliance of the patient, patient decision, physician decision, pregnancy, clinical progression, or sponsor decision. Patient may discontinue treatment due to non-target lesion progression or appearance of new lesion. Response evaluable population includes eligible patients who have at least one post-baseline assessment of tumor response. Best overall response per investigator assessment.

1L, first line; 2L, second line; BEMPEG, bempegaldesleukin; I-O, immunotherapy; NIVO, nivolumab; NSCLC, non-small cell lung carcinoma; PD-L1, programmed death ligand-1; RCC, renal cell carcinoma; RECIST, Response Evaluation Criteria In Solid Tumors.



Figure S4: Neutrophils, lymphocytes, and neutrophil-to-lymphocyte ratio over time by response. Mean neutrophil and lymphocyte concentrations using standard hematology (complete blood count with differential). Patients received various dose combinations of BEMPEG plus NIVO in cycle 1, and BEMPEG at 0.006 mg/kg plus NIVO 360 mg q3w in cycle 1–11. Best overall clinical response by RECIST v1.1.

BEMPEG, bempegaldesleukin; CR, complete response; NIVO, nivolumab; PD, progressive disease.; PR, partial response; RECIST, Response Evaluation Criteria In Solid Tumors; SD, stable disease.



Figure S5: Intratumoral expression of immune pathway and cell-type score with BEMPEG plus NIVO. Gene analysis using the NanoString nSolver platform was performed pre- and on-treatment (week 3) for all patients with available tumor samples (19 baseline and 9 on-treatment tumor biopsies) to assess the effect of BEMPEG plus NIVO on selected immune pathways (A) and cell types (B). Unpaired t-test; \* P≤0.05; \*\* P≤0.01; \*\*\* P≤0.001. Genes shown remained significant with multiple testing correction (Benjamini­–Hochberg correction).



Figure S6: Treatment with BEMPEG plus NIVO increases tumor immune infiltration and PD-L1 protein expression (by immunohistochemistry). Compared with matched baseline biopsies, week 3 biopsies show (A) increases in intratumoral CD8+ T cells (n=14), and (B) increased tumor PD-L1 expression (n=13). (C) Changes in CD8+ and PD-L1 in tumor samples from baseline to week 3 according to best overall clinical response: PR, SD, PD (RECIST v1.1).

1L, first line; 2L, second line; BEMPEG, bempegaldesleukin; BL, baseline; NS, not significant; NIVO, nivolumab; NSCLC, non-small cell lung carcinoma; PD-L1, programmed death-ligand 1; PD, progressive disease; PR, partial response; RCC, renal cell carcinoma; SD, stable disease; WK, week.

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Figure S7. Combination therapy with BEMPEG plus NIVO does not affect B cells in the tumor.Fourteen matched pre- and on-treatment tumor biopsies. Percentage of CD19+ B cells within total CD45+ cells and in total live cells before and after treatment (week 3) with BEMPEG plus NIVO for all patients (A) and split by response (B) Percentage of CD19+ B cells within total CD45+ cells and in total live cells at baseline according to response. Best overall response (RECIST v1.1) was complete or partial response for responders or stable disease or progressive disease for non-responders. Unpaired t-test with significance value of P<0.05.

BEMPEG, bempegaldesleukin; BL, baseline; NIVO, nivolumab; RECIST, Response Evaluation Criteria In Solid Tumors; wk3, week 3 of treatment.

# Supplemental Tables

## Table S1: Treatment exposure

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Melanoma****n=11** | **1L RCC****n=14** | **2L RCC****(I-O naïve)****n=8** | **NSCLC****(I-O naïve)****n=5** | **Total**N=38 |
| **Total duration of exposure, months** |
|  Mean (SD) | 11.6 (7.44) | 12.4 (6.67) | 8.2 (6.48) | 12.6 (9.17) | 11.3 (7.11) |
|  Median | 14.1 | 12.8 | 6.4 | 16.9 | 13.3 |
|  Q1, Q3 | 2.1, 16.6 | 5.2, 18.3 | 2.6, 15.2 | 3.5, 19.5 | 3.7, 16.9 |
|  Min, max | 1, 21 | 2, 22 | 1, 16 | 2, 21 | 1, 22 |
| **Total number of cycles** |
|  n | 11 | 14 | 8 | 5 | 38 |
|  Mean (SD) | 15.4 (9.47) | 16.7 (9.90) | 12.8 (10.94) | 13.4 (10.31) | 15.1 (9.76) |
|  Median | 20.0 | 15.0 | 9.0 | 10.0 | 15.0 |
|  Q1, Q3 | 3.0, 22.0 | 8.0, 26.0 | 3.5, 24.0 | 7.0, 18.0 | 6.0, 22.0 |
|  Min, max | 3, 30 | 3, 37 | 1, 28 | 3, 29 | 1, 37 |
| **Number of cycles, n (%)** |
|  <6 | 3 (27.3) | 1 (7.1) | 3 (37.5) | 1 (20.0) | 8 (21.1) |
|  ≥6 | 8 (72.7) | 13 (92.9) | 5 (62.5) | 4 (80.0) | 30 (78.9) |

I-O, immunotherapy; NSCLC, non-small cell lung carcinoma; RCC, renal cell carcinoma; SD, standard deviation.

## Table S2: TEAEs (all-Grade in ≥10% of patients)

|  |  |  |
| --- | --- | --- |
| **Preferred terma** | **All patients(N=38)** | **Patients treated at the RP2D(n=25)** |
| At least one TEAE | 38 (100.0) | 25 (100.0) |
| Flu-like symptomsb | 33 (86.8) | 20 (80.0) |
| Rashc | 31 (81.6) | 21 (84.0) |
| Fatigue | 28 (73.7) | 19 (76.0) |
| Arthralgia | 22 (57.9) | 14 (56.0) |
| Diarrhea | 22 (57.9) | 13 (52.0) |
| Pruritus | 22 (57.9) | 14 (56.0) |
| Cough | 20 (52.6) | 15 (60.0) |
| Decreased appetite | 19 (50.0) | 12 (48.0) |
| Headache | 19 (50.0) | 12 (48.0) |
| Nausea | 19 (50.0) | 13 (52.0) |
| Nasal congestion | 18 (47.4) | 10 (40.0) |
| Edema peripheral | 17 (44.7) | 11 (44.0) |
| Dry skin | 14 (36.8) | 8 (32.0) |
| Hypotension | 14 (36.8) | 9 (36.0) |
| Constipation | 13 (34.2) | 8 (32.0) |
| Vomiting | 13 (34.2) | 7 (28.0) |
| Abdominal pain | 12 (31.6) | 8 (32.0) |
| Back pain | 12 (31.6) | 8 (32.0) |
| Myalgia | 12 (31.6) | 9 (36.0) |
| Dizziness | 10 (26.3) | 7 (28.0) |
| Hypothyroidism | 10 (26.3) | 7 (28.0) |
| Peripheral sensory neuropathy | 10 (26.3) | 6 (24.0) |
| Face edema | 9 (23.7) | 5 (20.0) |
| Flushing | 8 (21.1) | 2 (8.0) |
| Insomnia | 8 (21.1) | 5 (20.0) |
| Pain in extremity | 8 (21.1) | 6 (24.0) |
| Dyspepsia | 7 (18.4) | 4 (16.0) |
| Dysphonia | 7 (18.4) | 5 (20.0) |
| Dyspnoea | 7 (18.4) | 6 (24.0) |
| Oropharyngeal pain | 7 (18.4) | 4 (16.0) |
| Dry mouth | 6 (15.8) | 4 (16.0) |
| Dysgeusia | 6 (15.8) | 5 (20.0) |
| Malaise | 6 (15.8) | 3 (12.0) |
| Musculoskeletal pain | 6 (15.8) | 3 (12.0) |
| Vision blurred | 6 (15.8) | 4 (16.0) |
| Infusion related reaction | 5 (13.2) | 4 (16.0) |
| Musculoskeletal chest pain | 5 (13.2) | 5 (20.0) |
| Upper respiratory tract infection | 5 (13.2) | 3 (12.0) |
| Weight decreased | 5 (13.2) | 3 (12.0) |
| Flank pain | 4 (10.5) | 1 (4.0) |
| Hyperglycemia | 4 (10.5) | 3 (12.0) |
| Neck pain | 4 (10.5) | 4 (16.0) |
| Non-cardiac chest pain | 4 (10.5) | 4 (16.0) |
| Productive cough | 4 (10.5) | 4 (16.0) |
| Stomatitis | 4 (10.5) | 2 (8.0) |
| Urinary tract infection | 4 (10.5) | 3 (2.0) |

All data are n (%).

aPatients are only counted once under each preferred term.

bIncludes the following preferred terms: chills, influenza-like illness, pyrexia.

cIncludes the following preferred terms: erythema, rash, rash erythematous, rash generalized, rash macular, rash maculo-papular, rash maculovesicular, rash papular, rash pruritic, rash pustular, rash vesicular, exfoliative rash.

Note: TEAE include the adverse events related to either bempegaldesleukin or nivolumab.

RP2D, recommended phase 2 dose; TEAE, treatment-emergent adverse event.

## Table S3: Changes in gene expression in tumor biopsies from baseline to week 3 (P<0.05)

|  |  |  |
| --- | --- | --- |
| **Gene** | **Fold change** | ***P*-valuea** |
| *TRAF3* | 1.66 | <0.001 |
| *STAT4* | 4.11 | <0.001 |
| *PIK3CD* | 1.91 | <0.001 |
| *MIF* | 0.47 | <0.001 |
| *KLRC1* | 3.61 | <0.001 |
| *TLR6* | 1.97 | <0.001 |
| *ITGAL* | 2.54 | <0.001 |
| *SH2D1A* | 3.41 | <0.001 |
| *CD96* | 3.04 | <0.001 |
| *TBX21* | 2.85 | <0.001 |
| *CD6* | 3.62 | <0.001 |
| *LCP1* | 2.09 | <0.001 |
| *BATF* | 3.89 | <0.001 |
| *CD247* | 3.19 | <0.001 |
| *TNF* | 2.17 | 0.0010 |
| *SPN* | 3.17 | 0.0011 |
| *CASP8* | 1.55 | 0.0011 |
| *SELPLG* | 2.47 | 0.0012 |
| *IL16* | 2.25 | 0.0012 |
| *MICB* | 1.91 | 0.0012 |
| *CD3E* | 3.19 | 0.0012 |
| *GZMA* | 2.65 | 0.0012 |
| *IL2RG* | 2.59 | 0.0013 |
| *IL2RB* | 3.35 | 0.0013 |
| *TARP* | 2.55 | 0.0014 |
| *PIK3CG* | 2.27 | 0.0015 |
| *LCK* | 2.73 | 0.0015 |
| *PDCD1LG2* | 2.18 | 0.0016 |
| *ZAP70* | 3.31 | 0.0017 |
| *CD3G* | 3.72 | 0.0017 |
| *CXCR3* | 2.50 | 0.0018 |
| *CD2* | 3.78 | 0.0018 |
| *INPP5D* | 2.00 | 0.0019 |
| *IKBKE* | 1.96 | 0.0020 |
| *JAK3* | 2.66 | 0.0023 |
| *BTK* | 2.04 | 0.0023 |
| *IL12RB1* | 2.53 | 0.0023 |
| *CD7* | 3.93 | 0.0023 |
| *ITK* | 3.83 | 0.0025 |
| *LYN* | 1.68 | 0.0025 |
| *FLT3LG* | 2.09 | 0.0026 |
| *TLR4* | 1.83 | 0.0027 |
| *MYD88* | 1.59 | 0.0028 |
| *CXCR6* | 4.72 | 0.0028 |
| *TNFRSF1B* | 2.35 | 0.0030 |
| *CASP3* | 1.42 | 0.0031 |
| *CD3D* | 2.76 | 0.0033 |
| *CD4* | 2.19 | 0.0035 |
| *GZMM* | 4.97 | 0.0036 |
| *FYN* | 1.73 | 0.0036 |
| *TNFSF13B* | 1.70 | 0.0036 |
| *JAK2* | 1.49 | 0.0037 |
| *CARD11* | 1.76 | 0.0038 |
| *CD48* | 2.33 | 0.0039 |
| *CASP1* | 1.48 | 0.0039 |
| *CR1* | 3.97 | 0.0039 |
| *KLRK1* | 2.99 | 0.0040 |
| *NFATC1* | 1.62 | 0.0040 |
| *CLEC4A* | 2.36 | 0.0041 |
| *PRF1* | 3.16 | 0.0046 |
| *CD33* | 3.23 | 0.0047 |
| *KLRG1* | 4.82 | 0.0048 |
| *POU2F2* | 2.22 | 0.0049 |
| *SLAMF6* | 4.54 | 0.0049 |
| *CTLA4* | 8.81 | 0.0050 |
| *PTPRC* | 2.07 | 0.0051 |
| *LILRB2* | 2.46 | 0.0052 |
| *GZMH* | 3.51 | 0.0053 |
| *APP* | 0.66 | 0.0055 |
| *CD244* | 4.06 | 0.0055 |
| *TNFRSF18* | 4.89 | 0.0056 |
| *NCF4* | 2.05 | 0.0057 |
| *LILRB3* | 4.16 | 0.0058 |
| *CD8A* | 2.42 | 0.0058 |
| *IRF8* | 2.24 | 0.0058 |
| *ICOS* | 4.34 | 0.0059 |
| *TLR8* | 2.55 | 0.0062 |
| *GPI* | 0.63 | 0.0064 |
| *ETS1* | 1.58 | 0.0064 |
| *KLRD1* | 3.68 | 0.0066 |
| *CCR5* | 2.56 | 0.0069 |
| *FOXP3* | 6.09 | 0.0069 |
| *IL10RA* | 1.88 | 0.0070 |
| *CX3CL1* | 0.36 | 0.0072 |
| *CD38* | 2.53 | 0.0074 |
| *CD5* | 3.51 | 0.0074 |
| *IL4R* | 1.28 | 0.0076 |
| *IL18R1* | 1.98 | 0.0077 |
| *ISG20* | 2.01 | 0.0080 |
| *LILRB1* | 2.04 | 0.0081 |
| *CYLD* | 1.39 | 0.0084 |
| *IL21R* | 3.82 | 0.0086 |
| *MTMR14* | 1.36 | 0.0087 |
| *TNFAIP3* | 2.32 | 0.0087 |
| *NOTCH1* | 1.35 | 0.0088 |
| *RUNX3* | 2.36 | 0.0088 |
| *NLRC5* | 1.84 | 0.0088 |
| *NLRP3* | 2.29 | 0.0090 |
| *CSF2RB* | 2.54 | 0.0093 |
| *CNOT10* | 1.28 | 0.0094 |
| *IL2RA* | 6.25 | 0.0096 |
| *TNFRSF8* | 4.25 | 0.0098 |
| *CCR2* | 2.20 | 0.0099 |
| *ILF3* | 0.80 | 0.0106 |
| *NOD1* | 1.40 | 0.0108 |
| *PPIA* | 0.62 | 0.0111 |
| *GZMB* | 4.20 | 0.0112 |
| *IRF1* | 1.70 | 0.0112 |
| *KLRB1* | 3.96 | 0.0116 |
| *ENTPD1* | 1.80 | 0.0117 |
| *NOD2* | 2.21 | 0.0118 |
| *CD53* | 1.78 | 0.0122 |
| *LAIR2* | 2.37 | 0.0125 |
| *RELB* | 1.48 | 0.0127 |
| *SELL* | 3.12 | 0.0127 |
| *ATG7* | 1.57 | 0.0129 |
| *CCL5* | 2.32 | 0.0137 |
| *CXCL14* | 0.17 | 0.0138 |
| *IFNAR2* | 1.28 | 0.0138 |
| *LTB* | 3.53 | 0.0139 |
| *ITGA4* | 1.50 | 0.0139 |
| *MAP3K1* | 1.24 | 0.0141 |
| *LILRA5* | 2.49 | 0.0141 |
| *LTA* | 3.68 | 0.0142 |
| *IL10* | 3.37 | 0.0143 |
| *CD99* | 1.48 | 0.0152 |
| *CD37* | 2.33 | 0.0152 |
| *CD28* | 5.45 | 0.0153 |
| *NFATC2* | 1.72 | 0.0154 |
| *TIGIT* | 3.62 | 0.0157 |
| *TLR5* | 1.81 | 0.0160 |
| *TANK* | 1.22 | 0.0160 |
| *TNFRSF4* | 2.46 | 0.0161 |
| *CYBB* | 2.00 | 0.0162 |
| *HCK* | 1.80 | 0.0163 |
| *IFNA7* | 0.00 | 0.0166 |
| *NFKB2* | 1.46 | 0.0170 |
| *CLEC7A* | 1.72 | 0.0172 |
| *TGFB2* | 0.42 | 0.0175 |
| *LY96* | 1.56 | 0.0179 |
| *ICAM3* | 1.69 | 0.0183 |
| *IL18RAP* | 5.29 | 0.0184 |
| *LILRA1* | 2.88 | 0.0189 |
| *FADD* | 1.56 | 0.0201 |
| *C3AR1* | 1.80 | 0.0202 |
| *ARG2* | 0.04 | 0.0214 |
| *LY86* | 1.90 | 0.0219 |
| *AMICA1* | 2.76 | 0.0223 |
| *TXK* | 3.35 | 0.0223 |
| *HLA-DQB1* | 3.20 | 0.0228 |
| *IFNG* | 3.81 | 0.0231 |
| *GNLY* | 2.14 | 0.0234 |
| *ITGB2* | 1.96 | 0.0239 |
| *CTSS* | 1.94 | 0.0240 |
| *CD40LG* | 4.22 | 0.0241 |
| *IKBKB* | 1.58 | 0.0243 |
| *CCR4* | 5.74 | 0.0245 |
| *TLR3* | 0.41 | 0.0245 |
| *CD58* | 1.24 | 0.0248 |
| *MAPK1* | 0.84 | 0.0253 |
| *TCF7* | 2.96 | 0.0256 |
| *CD86* | 1.91 | 0.0262 |
| *IRF4* | 2.56 | 0.0263 |
| *BTLA* | 3.69 | 0.0265 |
| *PDCD1* | 3.62 | 0.0266 |
| *IRF5* | 1.64 | 0.0267 |
| *COL3A1* | 3.20 | 0.0269 |
| *NCR1* | 5.24 | 0.0270 |
| *C1QA* | 1.78 | 0.0273 |
| *CD163* | 1.97 | 0.0273 |
| *IL7R* | 2.35 | 0.0274 |
| *NFATC3* | 1.24 | 0.0279 |
| *CD24* | 0.39 | 0.0281 |
| *HLA-DQA1* | 2.60 | 0.0283 |
| *KLRC2* | 4.80 | 0.0292 |
| *EOMES* | 2.43 | 0.0294 |
| *SOCS1* | 1.62 | 0.0295 |
| *CSF1R* | 2.04 | 0.0307 |
| *IRF2* | 1.29 | 0.0309 |
| *LAG3* | 3.63 | 0.0313 |
| *TNFSF8* | 1.67 | 0.0321 |
| *MAP3K5* | 1.56 | 0.0326 |
| *CD8B* | 2.11 | 0.0327 |
| *CARD9* | 2.10 | 0.0328 |
| *CD79B* | 2.75 | 0.0336 |
| *LY9* | 3.52 | 0.0340 |
| *PYCARD* | 1.41 | 0.0340 |
| *CD84* | 1.72 | 0.0340 |
| *FCGR2A* | 1.74 | 0.0349 |
| *ITGA6* | 0.68 | 0.0362 |
| *CXCL9* | 3.04 | 0.0372 |
| *ELK1* | 1.55 | 0.0373 |
| *TGFB1* | 1.40 | 0.0383 |
| *TLR7* | 1.85 | 0.0387 |
| *ERCC3* | 0.87 | 0.0388 |
| *SPA17* | 0.60 | 0.0389 |
| *CCND3* | 1.45 | 0.0403 |
| *REL* | 1.44 | 0.0405 |
| *CXCL13* | 3.24 | 0.0407 |
| *G6PD* | 1.31 | 0.0414 |
| *BST1* | 1.43 | 0.0417 |
| *ZNF143* | 1.20 | 0.0420 |
| *ECSIT* | 0.67 | 0.0427 |
| *CD1D* | 2.49 | 0.0432 |
| *ITGA2* | 0.59 | 0.0438 |
| *C1QB* | 1.82 | 0.0440 |
| *SLAMF7* | 2.14 | 0.0442 |
| *TNFSF12* | 1.33 | 0.0451 |
| *IL17RA* | 1.41 | 0.0455 |
| *DEFB1* | 0.10 | 0.0457 |
| *CAMP* | 9.51 | 0.0459 |
| *EPCAM* | 0.38 | 0.0474 |
| *SIGIRR* | 1.38 | 0.0474 |
| *FCGR3A* | 1.61 | 0.0475 |
| *CCR7* | 9.40 | 0.0478 |
| *NCAM1* | 0.34 | 0.0486 |
| *MRC1* | 2.50 | 0.0492 |
| *CD80* | 2.11 | 0.0493 |

aP values calculated using a one-sided t-test.

Biopsies (baseline: 19; on-treatment: 9) were analyzed for changes in gene expression across 760 genes. BEMPEG plus NIVO treatment significantly (P≤0.05, unadjusted for multiplicity) changed expression levels in 218 genes, leading to up-regulation in 197 genes, and down-regulation in 21 genes.

# Supplemental Material

## Definition of dose-limiting toxicity (DLT)

A DLT was defined as any Grade ≥3 non-hematologic adverse event (AE), deemed related or possibly related to study drug, occurring within the DLT window that does not resolve to Grade 1 or baseline within 7 days; any Grade 4 drug-related hematologic AE that is clinically significant; any Grade 4 nausea or vomiting; any Grade ≥2 myocarditis or uveitis; any Grade ≥3 pneumonitis or neurotoxicity; or any Grade ≥3 drug-related hypotension lasting >48 hours following drug administration, cytokine release syndrome, capillary leak syndrome, pulmonary edema, symptomatic hypereosinophilic syndrome, or qualifying drug-induced liver injury.

The following Grade 3 or 4 AEs should not be considered a DLT: endocrinopathy AEs, such as adrenal insufficiency, adrenocorticotropic hormone deficiency, hyper- or hypothyroidism, that resolve or are adequately controlled with physiologic hormone replacement (corticosteroids, thyroid hormones) or glucose intolerance managed with glucose-controlling agents; asymptomatic amylase or lipase elevations; lymphopenia <14 days in duration or not associated with clinical manifestations; electrolyte imbalances/abnormalities that are not associated with clinical sequelae and are corrected with supplementation/appropriate management within 72 hours of their onset; tumor flare defined as local pain, irritation, or rash localized at sites of known or suspected tumor; Grade 3 nausea or vomiting that can be medically managed to ≤Grade 2 within 72 hours; Grade 3 hypotension during cycles 1 or 2 that lasts ≤48 hours post-dose; fatigue that improves to ≤Grade 2 within 7 days.

## Biomarker methodology

### Tumor biopsies

Tumor biopsies (archival and fresh) were obtained at baseline and after cycle one between days 15–21 for: flow cytometry analysis of regulatory T cells (Tregs), CD8+ and CD4+ T cells, and NK cells (fresh only); immunohistochemistry (IHC) analysis of immune markers, including PD-L1 and CD8 expression; gene expression profiling; and T-cell receptor (TCR) sequencing.

### Flow cytometry

To characterize CD4+, CD8+ T cells, natural killer cells, and Tregs in the blood and in the tumor, peripheral blood mononuclear cells and tumor tissue were stained and analyzed using flow cytometry as previously described (Bentebibel S, *et al.* A first-in-human study and biomarker analysis of NKTR-214, a novel IL2βγ-biased cytokine, in patients with advanced or metastatic solid tumors. *Cancer Discov* 2019; 9:711–21).

### Immunohistochemistry

Multiplex immunofluorescence staining was performed using Vectra® (PerkinElmer, Richmond, CA, USA). IHC analysis for immune markers, included PD-L1 (Mosaic Laboratories, Lake Forest, CA, USA; rabbit anti-human antibody clone 28-8) and CD8 (mouse anti-human antibody clone C8/144B, Mosaic Laboratories) expression. Tumor tissue was tested centrally to determine PD-L1 expression: PD-L1-positive tumors were defined as staining on ≥1% of tumor cells, provided a minimum of 100 tumor cells were evaluable in the sample. In the case of insufficient tumor tissue, local pathology data were used to assess baseline PD-L1 status. Tumor T-cell infiltration was determined at baseline and on-treatment (week 3), with lower baseline T-cell infiltration defined as containing <150 cells/mm2, the median number of CD8+ T-cells observed at baseline.

### NanoString nCounter gene expression

NanoString nCounter® gene expression assay was performed on RNA extracted from tumor biopsies using the RNeasy Micro Kit (Qiagen) followed by hybridization with code sets. Samples were scanned using the nCounter® Digital Analyzer as per the manufacturer's instructions (NanoString Technologies, Seattle, WA). Gene expression was analyzed using Human PanCancer Immune Profiling Panel. The expression levels of each gene were normalized to those of control genes using a customary software (Precision for Medicine) and corrected for false discovery using the Benjamini–Hochberg method. Pathway and cell type gene analysis were performed and normalized using NanoString's software nSolver v3.0.22 with the Advanced Analysis Module v2.0.

### TCR sequencing analysis

Fresh tumor tissue was used to evaluate T-cell fraction and clonality using TCR sequencing as previously described (Bentebibel S, *et al.* A first-in-human study and biomarker analysis of NKTR-214, a novel IL2βγ-biased cytokine, in patients with advanced or metastatic solid tumors. *Cancer Discov* 2019; 9:711–21).

## Hydration guidelines

Important safety information and hydration instructions are to be provided to patients.

Consideration should be given to withholding antihypertensive medications including diuretics, as well as other drugs with hypotensive properties (e.g., alpha blockers for benign prostatic hyperplasia), particularly when therapy involves multiple antihypertensive drugs and classes other than thiazide diuretics. Study patients who are on medications with antihypertensive effects for the treatment of coronary artery disease (egg, beta-blockers, calcium channel blockers, nitrates, etc.) should be able to temporarily withhold these drugs. If withholding antihypertensive medications, withhold no less than 12 hours and no more than 48 hours prior to each dose of BEMPEG. Antihypertensive medications may be reinstituted in between doses of BEMPEG at any time as clinically indicated (e.g. based on blood pressure monitoring result).

Adequate hydration mitigates the development of hypotension associated with BEMPEG administration. Unless medically contraindicated, patients should be given at least one liter of IV fluids on the day of each dosing of BEMPEG. For the next 3 days (days 2–4) after BEMPEG administration, instruct patients to drink at least 2 liters per day of self-administered oral hydration. Advise patients to restrain from strenuous activity and avoid long hot showers and saunas for days 1 to 4 of every cycle. Per clinical judgment, IV fluids may be administered in any cycle. The Investigator may decide to forego administering IV fluids to a patient if this is deemed in the best interest of the patient (e.g. evidence of fluid overload).

Patients with pre-existing adrenal impairment requiring corticosteroid supplementation may be at increased risk for hypotensive episodes during treatment with BEMPEG. For these patients, it may be necessary to provide additional corticosteroid support.