**Supplementary materials:**

**Single-cell 32-plex functional proteomic profiling**

Cryopreserved bone marrow samples were thawed and cultured in complete RPMI medium (Fisher Scientific) supplemented with 10 ng/mL IL-2 (Biolegend) at a density of 1 X $10^6$ cells/mL in a 37°C, 5% $CO_2$ incubator overnight. After overnight recovery, viable T cells were purified from dead cells using Ficoll-Paque Plus medium (GE Healthcare). CD8+ then CD4+ T-cell subsets were sequentially enriched using anti-CD8 or anti-CD4 MicroBeads (Miltenyi Biotec) and resuspended in complete RPMI media at a density of 1 x 106/mL. An aliquot of 100 µL of CD4+ or CD8+ T cell suspension was seeded into a well of 96-well flat-bottom plate precoated with anti-human CD3 (clone OKT3, Thermo Fisher/Invitrogen , 10 ug/ml in PBS at 4°C, O/N) with a supplement of soluble anti-human CD28 (clone CD28.2, Thermo Fisher/Invitrogen) at a final concentration of 5 µg/mL. After cultured at 37°C, 5% CO2 for 24 hours, the cells were stained with Alexa Fluor 647-conjugated anti-CD4 or CD8 antibody (BioLegend) at room temperature for 10 minutes, rinsed once with complete RPMI and then resuspended in complete RPMI medium at a density of 1 x $10^6$/mL. Approximately 30 µL of CD4+ or CD8+ T cell suspension was loaded into an IsoCode Chip (IsoPlexis) and incubated at 37°C, 5% CO2 for additional 16 hours. Following this final incubation, subsequently secreted proteins from ~1000 single T cells were captured by the 32-plex antibody barcoded chip and analyzed by backend fluorescence ELISA-based assay. Polyfunctionality of T cells defined as a cell co-secreting 2+ cytokines was analyzed by the IsoSpeak software across the five functional groups(Supplementary Table 2).The polyfunctional strength Index (PSI) of T cells was computed using a pre-specified formula, defined as the percentage of polyfunctional cells, multiplied by the sum of the mean fluorescence intensity (MFI) of the proteins secreted by those cells[7-10,19,21,22].

$$PSI_{sample} = (\%\ polyfunctional\ cells\ in\ sample) \sum_{i=1}^{32} MFI\ of\ secreted\ protein\ i\ of\ the\ polyfunctional\ cells$$

The functional groups of T cells were deconvoluted and visualized by 3D t-Distributed Stochastic Neighbor Embedding (3D-tSNE) and heatmap visualizations[19-22]. 3D t-SNE is a nonlinear dimensionality reduction tool used for visualizing multi-dimensional data in low-dimensional space (2D/3D) relying on computations based on algebraic topology and Riemannian geometry[20]. Briefly, as the raw MFI data feeds into the t-SNE algorithm and is subsequently transformed/reduced, it calculates similarities between data points and then tries to optimize where the data point would end up in this 3D space. 3D t-SNE of all single cells was

analyzed in the IsoSpeak software by using the following hyperparameters: theta: 0.5; perplexity: 50; maximum iterations: 1000.

**Supplementary Table 1**

| **Characteristic** | Overall, N = 16 | NR, N = 11 | Resp., N = 5 | p-value1 |
|---|---|---|---|---|
| **Gender, n/N (%)** | | | | >0.9 |
| F | 4/16 (25%) | 3/11 (27%) | 1/5 (20%) | |
| M | 12/16 (75%) | 8/11 (73%) | 4/5 (80%) | |
| **Age (years)** | 67.2+/-11.9 | 66.5+/-12.2 | 68.6+/-12.4 | >0.9 |
| **Cytogenetics, n/N (%)** | | | | >0.9 |
| Complex, -5/-7 | 8/16 (50%) | 5/11 (45%) | 3/5 (60%) | |
| Diploid | 1/16 (6.2%) | 1/11 (9.1%) | 0/5 (0%) | |
| Miscellaneous | 7/16 (44%) | 5/11 (45%) | 2/5 (40%) | |
| **Prior Hypomethylating Agents** | | | | |
| Yes | 8/16 (50%) | 7/11 (63.6%) | 1/5 (20%) | |
| No | 8/16 (50%) | 4/11 (36.3%) | 4/5 (80%) | |
| **Total Nivolumab Doses Mean+/-SD** | 11.0+/-9.8 | 5.8+/-3.4 | 22.4+/-9.8 | 0.004 |
| **Bone marrow blasts, Mean+/-SD** | 44.1+/-29.3 | 44.9+/-27.5 | 42.4+/-36.4 | 0.7 |
| **Median Time to response (months)** | N/A | N/A | 4.07 [0.9-12.6] | N/A |
| **Median Overall Survival (months)** | 6 [2.4-21.5] | 5.7 [2.4-7.6] | 14.8 [9.4 – 21.5] | <0.001 |
| **Median Event Free Survival (months)** | 4.6 [2.4-18.3] | 4 [2.4-7.6] | 12.6 [9.1-18.3] | <0.001 |
| **Median Duration of Response (months)** | N/A | NA+/-NA | 5.2 [0.5-14.7] | N/A |

**Supplementary Table 2**

| Functional Groups | Analytes |
|---|---|
| Effector | Granzyme B, TNF-a, IFN-g, MIP-1a, Perforin, TNF-b |
| Stimulatory | GM-CSF, IL-2, IL-5, IL-7, IL-8, IL-9, IL-12, IL-15, IL-21 |
| Chemoattractive | CCL-11, IP-10, MIP-1b, RANTES |

| | |
|---|---|
| Regulatory | IL-4, IL-10, IL-13, IL-22, sCD137, sCD40L, TGF-b1 |
| Inflammatory | IL-6, IL-17A, IL-17F, MCP-1, MCP-4, IL-1b |