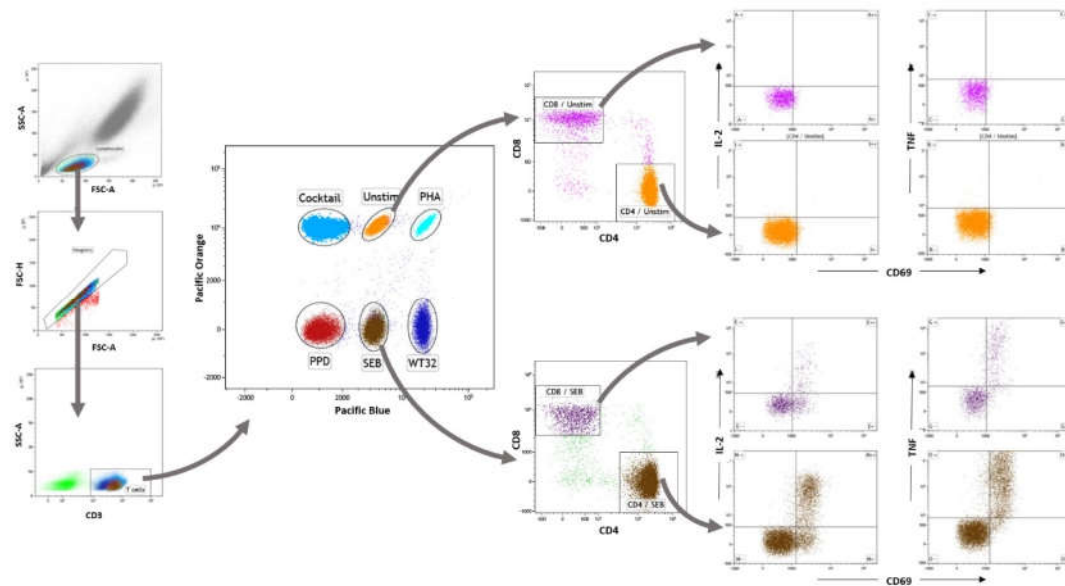
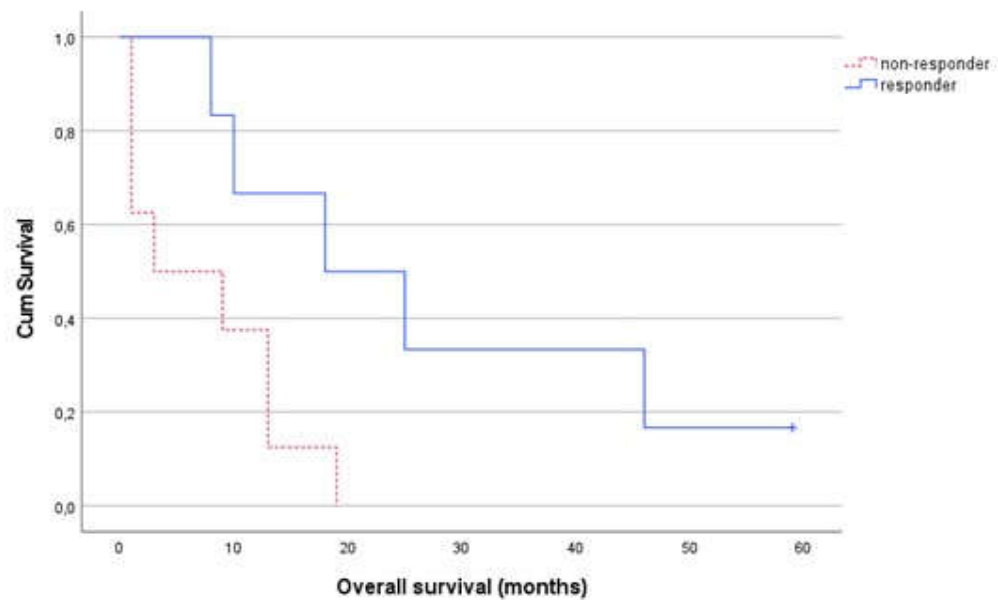


## Supplementary Figures and Tables



**Supplementary Figure S1.** Representative gating strategy of quantifying cytokine-expressing CD4<sup>+</sup> and CD8<sup>+</sup> T-cells using fluorescent cell barcoding (FCSB) technique. Peripheral blood lymphocytes were identified and gated based on forward (FSC) and side (SSC) scatter. After excluding doublets in the FSC-H/FSC-A plot, CD3<sup>+</sup> T cells were selected and analyzed for their Pacific Blue and Pacific Orange fluorescence intensity to identify the sample origin of stimulated cells. In this example, unstimulated and SEB stimulated CD4<sup>+</sup> and CD8<sup>+</sup> T cells were evaluated for the expression of cytokines (IL-2 and TNF) and activation marker (CD69). Gates for the expression of cytokine and CD69 were set based on unstimulated cells and applied uniformly to stimulated cells.



**Supplementary Figure S2.** Overall survival. Overall survival in months between responders ( $>$ median PFS) and non-responders ( $\leq$ median PFS). Cut-off for overall survival was last date of visit before 1 July 2024.

**Supplementary Table S1.** Baseline characteristics

		<b>Non-responders (N = 8)</b>	<b>Responders (N = 6)</b>
Patient	Age (years)	71 [52 – 85]	63 [52 – 69]
	Sex (female)	1 (13)	1 (17)
	Tumor histology		
	AC	5 (63)	5 (84)
	SCC	3 (38)	1 (17)
	Tumor Stage		
	IVA	2 (25)	2 (33)
	IVB	6 (75)	4 (67)
	ECOG PS		
	0	2 (25)	2 (33)
	1	6 (75)	4 (67)
	Smoking status		
	Never	1 (13)	-
	Former	7 (88)	3 (50)
	Current	-	3(50)
Alcohol usage (yes)		6 (75)	4 (67)
Lymphocyte count	Lymphocytes (10 <sup>9</sup> /L)	1.53 [0.87 – 2.16]	1.69 [1.37 – 2.62]
	CD3 <sup>+</sup> T-cells (10 <sup>9</sup> /L)	1.14 [0.68 – 1.93]	1.35 [0.84 – 2.03]
	CD4 <sup>+</sup> T-cells (10 <sup>9</sup> /L)	0.64 [0.33 – 1.31]	0.77 [0.55 – 1.08]
	CD8 <sup>+</sup> T-cells (10 <sup>9</sup> /L)	0.34 [0.12 – 1.03]	0.50 [0.13 – 1.01]
	CD19 <sup>+</sup> T-cells (10 <sup>9</sup> /L)	0.08 [0.02 – 0.35]	0.12 [0.06 – 0.37]
	NK-cells (10 <sup>9</sup> /L)	0.14 [0.09 – 0.34]	0.27 [0.18 – 0.47]
	Neutrophils (10 <sup>9</sup> /L)	6.31 [3.63 – 15.58]	5.85 [3.81 – 12.38]
	NLR	5.16 [1.78 – 9.80]	4.05 [1.54 – 8.04]

Data is presented as median [range] or n (%). Non-responders had progressive disease as their best response, responders had stable disease or partial response as their best response. AC: Adenocarcinoma. SCC: Squamous cell carcinoma. ECOG PS: Eastern Cooperative Oncology Group performance score. N: number. NK-cells: Natural Killer cells. NLR: Neutrophile-to-lymphocyte ratio. No significant differences were observed  $p > 0.14$ .

**Supplementary Table S2.** Correlation between lymphocyte count and survival

	Overall Survival	Progression Free Survival
Lymphocytes (10 <sup>9</sup> /L)	0.71 [0.005]	0.35 [0.22]
CD3 <sup>+</sup> T-cells (10 <sup>9</sup> /L)	0.73 [0.003]	0.23 [0.41]
CD4 <sup>+</sup> T-cells (10 <sup>9</sup> /L)	0.31 [0.29]	0.31 [0.27]
CD8 <sup>+</sup> T-cells (10 <sup>9</sup> /L)	0.63 [0.02]	0.14 [0.63]
CD19 <sup>+</sup> T-cells (10 <sup>9</sup> /L)	0.09 [0.76]	0.24 [0.39]
NK-cells (10 <sup>9</sup> /L)	0.21 [0.46]	0.43 [0.13]
Neutrophils (10 <sup>9</sup> /L)	-0.33 [0.25]	-0.16 [0.58]
NLR	-0.55 [0.04]	-0.29 [0.31]

Correlation is measured with Spearman's rho [P-value]. NK-cells: Natural Killer cells. NLR: Neutrophil-to-lymphocyte ratio

**Supplementary Table S3.** Median percentages of responding (CD69<sup>+</sup>) CD4<sup>+</sup> and CD8<sup>+</sup> T – cells and cytokine production after stimulation.

<b>CD4<sup>+</sup> T – Cells (%)</b>	<b>Responders</b>	<b>Non-responders</b>	<b>Healthy controls</b>
<b>Stimulated with SEB</b>			
CD69 <sup>+</sup>	18.0 [6.5 – 29.9]	12.6 [1.2 – 18.6]	7.3 [2.8 – 31.0]
IL2 <sup>+</sup> CD69 <sup>+</sup>	17.1 [4.3 – 23.4]	12.5 [4.5 – 21.4]	7.4 [2.7 – 11.2]
IFN $\gamma$ <sup>+</sup> CD69 <sup>+</sup>	9.8 [0.7 – 15.7]	6.4 [0.6 – 20.3]	3.1 [1.1 – 8.1]
TNF $\alpha$ <sup>+</sup> CD69 <sup>+</sup>	22.5 [5.7 – 30.4]	15.4 [6.5 – 31.7]	9.6 [4.3 – 16.9]
<b>Stimulated with aCD3</b>			
CD69 <sup>+</sup>	11.7 [1.37 – 31.0]	6.3 [0.1 – 25.9]	5.4 [0.0 – 17.6]
IL2 <sup>+</sup> CD69 <sup>+</sup>	1.75 [0.0 – 9.1]	0.6 [0.0 – 16.4]	0.0 [0.0 – 0.8]
IFN $\gamma$ <sup>+</sup> CD69 <sup>+</sup>	1.4 [0.0 – 5.2]	0.6 [0.0 – 19.4]	0.0 [0.0 – 1.4]
TNF $\alpha$ <sup>+</sup> CD69 <sup>+</sup>	10.3 [0.1 – 26.6]	6.2 [0.6 – 35.8]	2.5 [1.0 – 13.2]
<b>Stimulated with PHA</b>			
CD69 <sup>+</sup>	68.2 [33.7 – 78.2]	46.0 [17.7 – 82.4]	61.5 [12.4 – 84.4]
IL2 <sup>+</sup> CD69 <sup>+</sup>	29.0 [21.9 – 40.3]	21.7 [18.1 – 36.50]	29.2 [18.6 – 41.1]
IFN $\gamma$ <sup>+</sup> CD69 <sup>+</sup>	11.8 [1.9 – 27.5]	9.4 [0.9 – 67.6]	11.9 [6.0 – 30.2]
TNF $\alpha$ <sup>+</sup> CD69 <sup>+</sup>	47.0 [35.5 – 65.1]	46.4 [21.6 – 82.4]	46.5 [20.9 – 55.6]
<b>Stimulated with Cocktail</b>			
CD69 <sup>+</sup>	1.1 [0.5 – 2.0]	0.1 [0.0 – 1.3]	0.1 [0.0 – 2.5]
IL2 <sup>+</sup> CD69 <sup>+</sup>	0.1 [0.0 – 0.5]	0.1 [0.0 – 1.1]	0.0 [0.0 – 0.6]
IFN $\gamma$ <sup>+</sup> CD69 <sup>+</sup>	0.1 [0.0 – 0.2]	0.0 [0.0 – 0.3]	0.0 [0.0 – 0.5]
TNF $\alpha$ <sup>+</sup> CD69 <sup>+</sup>	0.1 [0.0 – 0.3]	0.1 [0.0 – 2.3]	0.0 [0.0 – 1.0]
<b>Stimulated with PPD</b>			
CD69 <sup>+</sup>	0.6 [0.0 – 3.1]	0.3 [0.0 – 2.5]	0.3 [0.0 – 1.7]
IL2 <sup>+</sup> CD69 <sup>+</sup>	0.0 [0.0 – 1.4]	0.2 [0.0 – 0.9]	0.0 [0.0 – 0.3]
IFN $\gamma$ <sup>+</sup> CD69 <sup>+</sup>	0.0 [0.0 – 0.3]	0.0 [0.0 – 0.5]	0.0 [0.0 – 0.4]
TNF $\alpha$ <sup>+</sup> CD69 <sup>+</sup>	0.0 [0.0 – 2.6]	0.0 [0.0 – 1.2]	0.1 [0.0 – 0.8]
<b>CD8<sup>+</sup> T – Cells (%)</b>	<b>Responders</b>	<b>Non-responders</b>	<b>Healthy controls</b>
<b>Stimulated with SEB</b>			
CD69 <sup>+</sup>	15.8 [6.1 – 25.8]	3.5 [0.7 – 12.0]	5.6 [0.5 – 17.1]
IL2 <sup>+</sup> CD69 <sup>+</sup>	8.8 [3.7 – 16.1]	2.9 [1.2 – 8.7]	4.7 [1.2 – 5.9]
IFN $\gamma$ <sup>+</sup> CD69 <sup>+</sup>	21.4 [3.4 – 25.2]	5.5 [2.9 – 31.2]	9.3 [2.4 – 16.4]
TNF $\alpha$ <sup>+</sup> CD69 <sup>+</sup>	22.5 [5.7 – 30.4]	8.0 [4.1 – 27.6]	10.4 [2.1 – 18.7]
<b>Stimulated with aCD3</b>			
CD69 <sup>+</sup>	17.6 [2.8 – 18.7]	7.6 [1.1 – 24.9]	6.5 [0.2 – 22.6]
IL2 <sup>+</sup> CD69 <sup>+</sup>	2.5 [0.0 – 6.1]	1.2 [0.1 – 5.7]	1.0 [0.0 – 2.9]
IFN $\gamma$ <sup>+</sup> CD69 <sup>+</sup>	6.4 [0.0 – 17.9]	6.3 [0.0 – 29.3]	4.1 [0.0 – 10.0]
TNF $\alpha$ <sup>+</sup> CD69 <sup>+</sup>	11.5 [0.7 – 12.8]	10.0 [1.9 – 23.1]	7.7 [1.5 – 14.3]
<b>Stimulated with PHA</b>			
CD69 <sup>+</sup>	45.6 [21.0 – 55.6]	18.3 [9.7 – 53.6]	31.1 [5.9 – 66.4]
IL2 <sup>+</sup> CD69 <sup>+</sup>	10.8 [6.9 – 18.8]	10.3 [4.7 – 19.4]	10.4 [4.7 – 17.7]
IFN $\gamma$ <sup>+</sup> CD69 <sup>+</sup>	29.3 [11.7 – 54.2]	38.5 [11.6 – 57.2]	26.7 [12.0 – 38.5]
TNF $\alpha$ <sup>+</sup> CD69 <sup>+</sup>	28.6 [13.6 – 37.7]	32.3 [17.3 – 57.2]	22.7 [14.0 – 38.8]
<b>Stimulated with Cocktail</b>			
CD69 <sup>+</sup>	0.7 [0.0 – 1.8]	0.2 [0.0 – 1.9]	0.2 [0.0 – 2.2]
IL2 <sup>+</sup> CD69 <sup>+</sup>	0.0 [0.0 – 0.3]	0.0 [0.0 – 0.5]	0.0 [0.0 – 0.6]
IFN $\gamma$ <sup>+</sup> CD69 <sup>+</sup>	0.0 [0.0 – 0.5]	0.0 [0.0 – 0.6]	0.0 [0.0 – 2.0]
TNF $\alpha$ <sup>+</sup> CD69 <sup>+</sup>	0.0 [0.0 – 0.2]	0.1 [0.0 – 1.0]	0.1 [0.0 – 2.2]

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**Stimulated with PPD**

CD69 <sup>+</sup>	0.5 [0.0 – 1.4]	0.3 [0.0 – 2.4]	0.2 [0.0 – 1.6]
IL2 <sup>+</sup> CD69 <sup>+</sup>	0.0 [0.0 – 0.0]	0.0 [0.0 – 0.1]	0.0 [0.0 – 0.2]
IFN $\gamma$ <sup>+</sup> CD69 <sup>+</sup>	0.0 [0.0 – 0.4]	0.0 [0.0 – 1.0]	0.0 [0.0 – 0.3]
TNF $\alpha$ <sup>+</sup> CD69 <sup>+</sup>	0.2 [0.0 – 1.2]	0.0 [0.0 – 1.1]	0.0 [0.0 – 1.2]

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Data is presented as median % [range]. aCD3: Anti-CD3. IFN $\gamma$ : Inteferon gamma. IL2: Interleukin 2. PHA: Phyto haemaglutine. PPD: Purified protein derivative Tuberculosis. SEB: Staphylococcal enterotoxin B. TNF $\alpha$ : Tumor necrosis factor alpha.

**Supplementary Table S4.** Serum cytokines

<b>Cytokine</b>	<b>Responders</b>	<b>Non-responders</b>
BAFF/TNF (pg/mL)	1347 [985 – 1791]	1559 [420 – 2113]
IL-2R (pg/ml)	1004 [683 – 2381]	1214 [178 – 6568]
IFN $\gamma$ (pg/ml)	2.47 [1.44 – 3.90]	2.64 [0.36 – 13.2]
CCL17/TARC (pg/ml)	600 [245 – 999]	496 [99.7 – 3228]
CXCL13 (pg/ml)	88.7 [35.7 – 233]	59.1 [25.0 – 88.1]
IL-6 (pg/ml)	3.01 [0.61 – 31.2]	6.69 [1.76 – 16.3]

Data is presented as median [range]. BAFF/TNF: B-cell activating factor/Tumor necrosis factor. CCL17/TARC: C-C Motif T-lymphocyte-associated protein 4/Thymus and activation regulated chemokine. CXCL13: C-X-C Motif Chemokine Ligand 13. IFN $\gamma$ : Interferon gamma. IL-2R: Interleukin 2 receptor. IL-6: Interleukin 6.