

Supplemental figure 1. Schematic overview of the clinical cohort.

The timeline for diagnosis of neurological (red) and non-neurological (black) irAEs and sampling is shown for the control (top) and n-irAE (bottom) groups. Time point 0 indicates the start of ICI treatment. The type of ICI treatment is indicated by color, and the length of the segments represents the follow-up duration.

Supplemental figure 2. Preprocessing of scRNA-seq data. (A) Distribution of quality control metrics: mitochondrial content (MTC), number of Unique Molecular Identifiers (UMIs), ribosomal content, and number of detected genes and the respective cutoffs applied to filter out low-quality cells. (B) UMAP projections of the patient origin, (C) initial cell type annotation by label transfer, (D) Seurat clustering, (E) mitochondrial content as a marker of low-quality cells, and (F) Azimuth prediction score. (G) Expression of cell type-specific markers. The average expression and the percentage of cells expressing the gene are reported via a color scale and the dot diameter. Cell type composition across (H) sample collection times, (I) patient groups, and (J) cancer entities.

Supplemental figure 3. Cell type abundance between the control and n-irAE groups.

(A) Abundance of the main immune cell types. (B-D) Cell type subpopulation abundances within the (B) CD4⁺ T cell, (C) CD8⁺ T cell and (D) B cell compartments. (E) Comparison of B cell maturity scores between samples collected after ICI-treatment with or without n-irAEs or (F) between patients without n-irAEs before and after ICI-treatment. P-values were calculated with classical (A-E) and partially matched Wilcoxon tests (F).

Supplemental figure 4. CD4⁺ T cell profile shifts associated with n-irAEs. (A) Pseudotime value distribution of CD4⁺ T cell subsets, (B) further stratified by groups (left) and per patient (right). (C) Pseudotime distribution of CD4⁺ T cell subsets per patient group. (D) Pseudotime,

AUCell *naïvety*, cytotoxicity, and exhaustion score median per group among the depicted CD4⁺ T cell populations. In (C,D), p-values were calculated with a Wilcoxon test. (E) Proportion of cells from n-irAE patients as a function of pseudotime calculated on a sliding window and 95% confidence interval after group assignment permutation (similar to **figure 3D**), after removing the control group outlier (Ctrl. 9). (F) Cellular composition of the CD4⁺ T cell compartment before and after ICI treatment in control patients.

Supplemental figure 5. CD8⁺ T cell profile shifts associated with n-irAEs. Slingshot pseudotime analysis of the CD8⁺ T cell compartment (main pseudotime branch). (A) Pseudotime value distribution among CD8⁺ T cell subsets. (B) Density of the pseudotime value per group (left) and per patient (right). (C) Pseudotime, AUCell *naïvety*, cytotoxicity, and exhaustion score median per group in displayed CD8⁺ T cell populations. In (G), p-values were calculated with a Wilcoxon test.

Supplemental figure 6. Phenotypic and transcriptional profile analysis of the most expanded n-irAE CD4⁺ CTL clones. (A) Volcano plot, displaying differentially expressed genes between the top 10 CD4⁺ CTL clones from n-irAE and control patients. Differentially expressed genes (adjusted p-value < 0.01 & absolute fold change > 1.5) upregulated in n-irAE and control patients are highlighted in red and yellow, respectively. (B) The first two principal components of a PCA at the clone-wise pseudobulk level (left) or in single cells (right) are shown for subsetted CD4⁺ CTLs. (C) Group (left) and patient origin (right) projected on the AUCell scores based on the genes shown in **figure 4A** for both control and n-irAE groups at the single-cell level. (D) Distribution of both control (left) and n-irAE (right) group AUCell scores calculated on single cells. P-values were calculated with a Wilcoxon test. (E) The top 20 Gene Ontology Biological Processes (GOBP) of the over-representation analysis in **figure 4F**.

53 **Supplemental figure 7. Transcriptomic profile analysis of the top 10 CD8⁺ TEM cell**
54 **clones. (A)** Heatmap showing the clone-wise pseudobulk expression of genes differentially
55 expressed in the control and n-irAE groups. **(B)** Gene Ontology Biological Processes (GOBP)
56 over-representation analysis of the n-irAE genes shown in **(A)**.

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59 **Online supplemental table 1. Resources and kits utilized in this study.**

Reagents	Vendor	Item Number/Version
Antibodies		
Alexa Fluor® 488 anti-human CD14 Antibody	BioLegend	Cat# 367130; RRID: AB_2721360
PE anti-human CD66b Antibody	BioLegend	Cat# 305106; RRID AB_2077857
Chemicals		
SYTOX™ Blue Dead Cell Stain, for flow cytometry	Thermo Fisher Scientific	Cat# S34857
Commercial Kits		
Chromium Next GEM Single Cell 5' Kit v2, 16 rxns	10x Genomics	PN-1000263
Library Construction Kit, 16 rxns	10x Genomics	PN-1000190
Chromium Single Cell Human TCR Amplification Kit, 16 rxns	10x Genomics	PN-1000252
Chromium Next GEM Chip K Single Cell Kit, 48 rxns	10x Genomics	PN-1000286
Dual Index Kit TT Set A, 96 rxns	10x Genomics	PN-1000215
Deposited data		
n-irAEs patient cohort (scRNAseq + TCR-seq); cell ranger outputs	This paper	GEO: GSE278119

n-irAEs patient cohort (scRNAseq + TCR-seq); fastq files	This paper	EGA (submission initiated)
Software and algorithms		
Reproducibility code	https://github.com/agiguelay/n_irAEs_reproducibility/	
CellRanger	10x Genomics	6.1.2
Seurat ¹⁵	Hao et al., 2021	4.3.0
edgeR ⁴⁹	Chen et al., preprint	3.42.4
DescTools	https://github.com/AndriSignorelli/DescTools/	0.99.54
AUCell ¹⁸	Aibar et al., 2017	1.24.0
ComplexHeatmap ⁵⁰	Gu et al., 2016	2.18.0
slingshot ¹⁷	Street et al., 2018	2.8.0
ggpubr	https://github.com/kassambara/ggpubr	0.6.0
clusterProfiler ⁵¹	Wu et al., 2021	4.6.0

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Online supplemental table 2. Clinical and treatment characteristics of study participants included in this study.
(Provided as a separate document due to table length.)

Online supplemental table 3. Summarized results of the differential gene expression analysis comparing top clones of CD4⁺ CTL from control and n-irAE patients.
(Provided as a separate document due to table length.)

70 *Patient and Public Involvement statement*

71 Patients and members of the public were actively involved at multiple stages of this research,
72 including the study design, management, and execution phases. Initial interviews with patients
73 informed the development of the research questions, ensuring alignment with patient priorities,
74 preferences, and experiences. Patients provided feedback throughout the study, particularly
75 during post-visit interviews, allowing continuous assessment and reduction of participant burden
76 regarding the intervention and associated time commitments. Additionally, patient input helped
77 optimize the scheduling and structure of questionnaires and examinations to further minimize
78 participation burden. We intend to continue engaging patients and the public in disseminating
79 the results, ensuring information shared is relevant, accessible, and impactful for patient
80 communities.

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