Deciphering Myocarditis: A Single-Cell Genomic Analysis of Immune-Related Adverse Events

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Abstract

The cellular dynamics and heterogeneity in human immune responses, particularly pertaining to adverse events, are intricate and have recently become accessible at a detailed level due to advances in single-cell genomics[1]. This paper underscores a focused exploration of immune-related adverse events, emphasizing conditions of myocarditis. Drawing inspiration from the Montreal Immune-Related Adverse Events (MIRAE) ICI-myocarditis project[2], we build on their foundational work by utilizing a distinct dataset from the University of Lady Davis[3]. By employing state-of-the-art tools like the Seurat toolkit[4], the seven-bridge platform[5], and the `scanpy` package for single-cell genomics[6], we have aimed to achieve comprehensive insights into cellular landscapes crucial to adverse events. Emphasizing data quality and robust analytical approaches, we provide insights into myocarditis that could prove invaluable for future clinical and research perspectives.

1 Introduction

The human immune response, especially as it relates to adverse events, presents a multifaceted landscape of cellular dynamics and heterogeneity. With the onset of single-cell genomics, it is now possible to probe these dynamics at an unprecedented resolution, potentially unlocking intricate patterns that traditional bulk analyses might overlook. Within this context, our study aims to explore the cellular underpinnings of immune-related adverse events, specifically focusing on the distinctions and subtleties that arise in different conditions of myocarditis.

Our motivation stems from the paradigm-shifting work undertaken in the Montreal Immune-Related Adverse Events (MIRAE) ICI-myocarditis project. Their investigation offered a comprehensive overview of immune cell subpopulations during various stages of myocarditis. Our endeavor seeks to build upon the groundwork laid by the MIRAE project, leveraging a unique dataset sourced from the University of Lady Davis.

To ensure the fidelity and robustness of our findings, we employed a methodological blueprint that emphasizes data quality, reproducibility, and state-of-the-art analytical approaches. Central to our methodology was the incorporation of the Seurat toolkit and the seven-bridge platform, both of which have been instrumental in advancing single-cell analyses.

This paper elucidates our analytical journey, from the initial data sourcing to intricate single-cell explorations, and offers insights into the cellular landscapes that are potentially pivotal in immune-related adverse events. Through our findings, we aspire to augment

the existing knowledge corpus on myocarditis and provide clinicians and researchers with nuanced perspectives that can guide future interventions and investigations.

2 Data

The foundational patient data for our study was sourced from the University of Lady Davis. These datasets, initially comprised of 5 distinct sets, collectively incorporated data samples from 10 patients. To ensure congruence with our research context, these samples were categorized into 4 unique stages, mirroring the classifications from the Montreal Immune-Related Adverse Events (MIRAE) ICI-myocarditis project: Baseline Myocarditis cases, irAEs (immune-related adverse events), Baseline controls, and Follow Up controls.

Our methodological approach commenced with data processing via the seven-bridge platform. This was followed by a transformative step where we transmuted the processed data into the h5ad format using the Seurat toolkit, a step akin to the multi-omics pipeline applied in the MIRAE study.

Recognizing the cardinal importance of data fidelity and coherence, we amalgamated the 3 h5ad files, and embarked on a series of rigorous filtration processes on the unified h5ad dataset. Mirroring the rigorous analytical approach from the MIRAE study where immune cell subpopulation profiling was carried out, we executed specific filters: genes detectable in less than 3 cells were removed, cells manifesting fewer than 200 expressed genes were excluded, and any cell where mitochondrial gene expression surpassed 20% was dismissed. These preprocessing maneuvers were imperative to vouchsafe data of impeccable quality, setting the stage for nuanced single-cell analysis. This, in turn, equipped us with the capability to delve deeply into the cellular dynamics and heterogeneity underlying the conditions being studied.

To visually expound on our data quality measures, we present a violin plot delineating three critical metrics: the count of expressed genes within the matrix, the aggregate counts for each cell, and the proportion of counts attributable to mitochondrial genes. A pertinent observation to note, given the data's origin from 5 disparate files, is the potential for a batch effect. This can inadvertently skew PCA analysis results. In an endeavor to obviate this, drawing inspiration from the meticulous techniques in the MIRAE study, we deployed the Harmony package to neutralize batch effects. The transformation is evidenced in two subsequent plots that vividly underscore the efficacy of batch effect mitigation.

3 Method

In the subsequent phase of our analysis, we adopted the `scanpy` package, a prevalent tool in single-cell genomics, to conduct gene clustering. The first step involved dimensionality reduction using principal component analysis (PCA) by employing the `sc.tl.pca` function. This aids in retaining the significant sources of variance in the data while discarding noise. To elucidate the importance of individual components, we visualized the variance ratio of the principal components with the pca variance ratio function. The visual interpretation from PCA invariably assists in determining the number of dimensions or principal components to be taken forward for further analysis. Based on the significant principal components derived from the PCA, we then projected our data onto a 2D space

using the UMAP (Uniform Manifold Approximation and Projection) visualization. This was facilitated by the `sc.pl.umap` function. UMAP serves as a powerful tool to visualize clusters in the data, potentially hinting at various cell types or states. Drawing parallels with the theoretical underpinnings of the MIRAE study, these methods enabled us to discern the intricate patterns and clusters embedded within our high-dimensional single-cell data.

4 Results

4.1 Cluster Generating and Top Gene

In the process of our analysis, a pivotal step was addressing potential batch effects, given that our data encompassed samples from multiple patients. Such effects can introduce spurious variability, potentially confounding our interpretations. We turned to the Harmony algorithm, which has been celebrated for its ability to harmonize single-cell datasets by aligning shared cellular states across diverse samples.

The efficacy of this batch effect removal is elucidated in the ensuing figure, which juxtaposes the data distributions before and after the Harmony intervention. This visual comparison underscores the enhancement in data uniformity, removing the potential biases introduced by individual patient datasets.

Subsequent to the batch correction, our attention pivoted to the clustering of the harmonized data. A primary outcome of this stage was the identification of the top 20 gene markers in each resultant cluster. By cross-referencing these markers with well-established PBMC (Peripheral Blood Mononuclear Cell) gene markers, we could assign meaningful biological labels to each cluster, facilitating a more intuitive interpretation of our results.

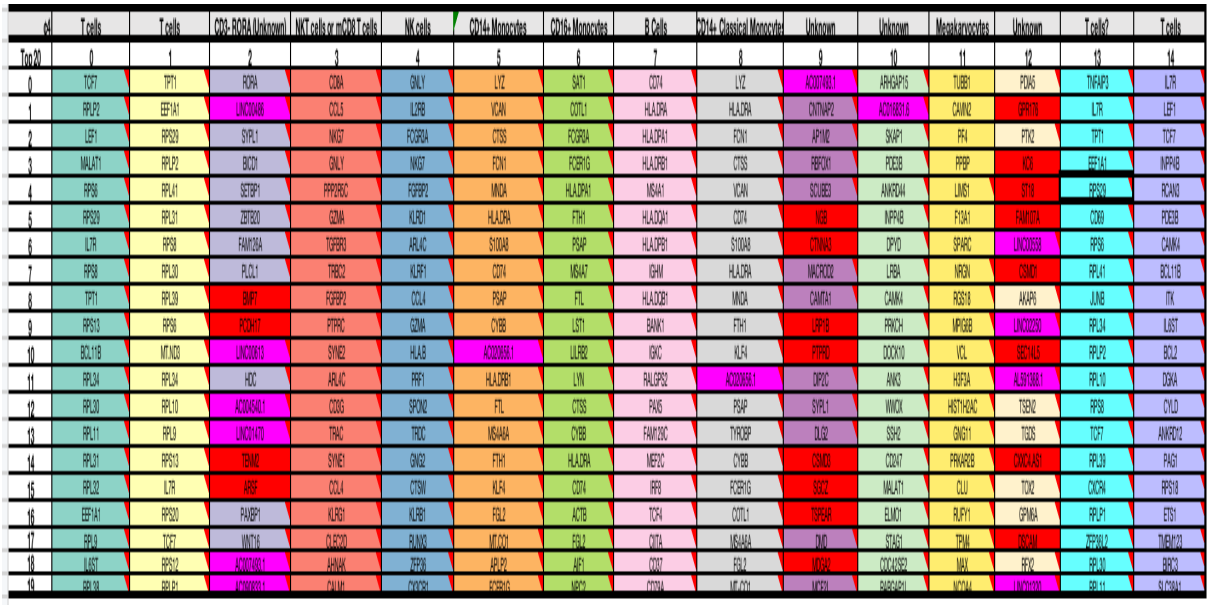
Furthermore, we noticed certain clusters exhibited highly similar gene expression profiles as shown in figure 1, based on the PBMC gene marker annotation, were identified as comparable cell types. To enhance the clarity and simplicity of our findings, these clusters were judiciously merged, providing a consolidated view of the cellular landscape in our dataset.

4.2 Preliminary Cluster Labeling and Gene Marker Validation

Upon the completion of data clustering, we generated preliminary labels for the various clusters based on the top gene markers identified. To rigorously validate these preliminary cluster labels, we employed the visualization of specific gene marker distributions across the clusters.

Using the `sc.pl.umap` function, we plotted the Uniform Manifold Approximation and Projection (UMAP) colored by selected critical gene markers, for example, `CD3D, CD3E, CD4, CD8A, NKG7, CD79A, IL2RA, FOXP3, IL10, CD14`, and `MARCH1`. Each gene marker set was chosen for its well-established role in characterizing specific cell types within the PBMC landscape.

For instance, the markers `CD3D, CD3E`, and `CD4` were used to validate clusters related to T-helper cells, while `CD8A, NKG7`, and `CD79A` were chosen for cytotoxic T cells and B cells, respectively. Similarly, markers like `IL2RA, FOXP3, IL10` helped in corroborating regulatory T-cell clusters, and `CD14, FCGR3A, ITGAM` were used for monocyte validation.



cl	T cells	T cells	CD2- RORA Unknown	NKT cells or CD8 T cells	NK cells	CD14+ Monocytes	CD16+ Monocytes	B Cells	CD14+ Classical Monocytes	Unknown	Unknown	Megakaryocytes	Unknown	T cells?	T cells
Top 20	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
0	TCF7	TPT1	RORA	CD8A	GNLY	LYZ	SAT1	CD74	LYZ	AC007163.1	ARHGAP15	TUBB1	PDK9	TNFAIP3	IL7R
1	RPLP2	EEF1A1	LINC00486	CCL5	IL2RB	VCAN	COTL1	HLA-DRA	HLA-DRA	CNTNAP2	AC010680.0	CAMK2	GPR176	IL7R	LEF1
2	LEF1	RPS29	SYPL1	NKG7	FCGR3A	CTSS	FCGR3A	HLA-DPA1	FCN1	AP1M2	SKAP1	PF4	PTK2	TPT1	TCF7
3	MALAT1	RPLP2	BCD1	GNLY	NKG7	FCN1	FCER1G	HLA-DPB1	CTSS	RBFOX1	PDE3B	PPBP	KCNQ1	EEF1A1	INPP4B
4	RPS6	RPL41	SETBP1	PPP2R2C	FGFBP2	MNDA	HLA-DRA1	MS4A1	VCAN	SCUBE3	ANKRD44	LIMS1	ST18	RPS29	ROAN3
5	RPS29	RPL21	ZBTB20	GZMA	KLRD1	HLA-DRA	FTH1	HLA-DQA1	CD74	NSB	INPP4B	F13A1	FAM107A	CD69	PDE3B
6	IL7R	RPS8	FAM129A	TGFBR3	ARL4C	S100A8	PSAP	HLA-DPB1	S100A8	CTNNA3	DPYD	SPARC	LINC00339	RPS6	CAMK4
7	RPS8	RPL20	PLCL1	TRBC2	KLRF1	CD74	MS4A7	IGHM	HLA-DRA	MACROD2	LRBA	NRGN	CSMD1	RPL41	BCL11B
8	TPT1	RPL29	BMP7	FGFBP2	CCL4	PSAP	FTL	HLA-DQB1	MNDA	CAMTA1	CAMK4	RGS18	AKAP6	JUNB	ITK
9	RPS13	RPS6	PCDH17	PTPRC	GZMA	CYBB	LST1	BANK1	FTH1	LRP1B	PRKCH	MYO5B	LINC02297	RPL34	LIMS1
10	BCL11B	MT-ND3	LINC00613	SYNE2	HLA-B	AC020656.1	LILRB2	IGKC	KLF4	FTPRD	DOCK10	VCL	SEC14L5	RPLP2	BCL2
11	RPL34	RPL34	HDC	ARL4C	PRF1	HLA-DRB1	LYN	RALGPS2	AC020656.1	DPP2C	ANK3	H3F3A	AL691388.1	RPL10	DOK4
12	RPL30	RPL10	AC004540.1	CD3G	SPON2	FTL	CTSS	PAX5	PSAP	SYPL1	WWOX	HIST1H2AC	TSPAN2	RPS8	CYLD
13	RPL11	RPL9	LINC01470	TRAC	TBX21	MS4A6A	CYBB	FAM129C	TYROBP	DLG2	SSH2	GNG11	TGDS	TCF7	ANKRD12
14	RPL31	RPS13	TENM2	SYNE1	GNG2	FTH1	HLA-DRA	MEF2C	CYBB	CSMD3	CD247	PRKAR2B	CXXC4-AS1	RPL39	PAG1
15	RPL32	IL7R	ARSB	CCL4	CTSW	KLF4	CD74	IRF8	FCER1G	SGCZ	MALAT1	CLU	TOX2	CXCR4	RPS18
16	EEF1A1	RPS20	PARP1	KLRG1	KLRB1	FBL2	ACTB	TCF4	COTL1	TSPEAR	ELMO1	RUFY1	GPNMB	RPLP1	ETS1
17	RPL9	TCF7	VMT10	OLFML3D	RUNX3	MT-CO1	FBL2	CIITA	MS4A6A	DMD	STAG1	TPM1	DOCK4	ZFP36L2	TMEM123
18	LIMS1	RPS12	AC007405.1	AHNAK	ZFP36L	ARL2	AIF1	CD37	FBL2	MYO1E	CDC42SE2	MAX	RFX2	RPL30	BRK3
19	RPL28	RPL21	AC092691.1	CNN1	CXCR3	FCER1G	MS4A7	CD79A	MT-CO1	MCTP1	PARP4P1	NCOA1	LINC02297	RPL11	SLC9A1

Figure 1: **top 20 genes for each cluster**

The following plots facilitated not only the verification of our preliminary cluster labels but also offered insightful qualitative evaluations of the relative expression levels of these critical genes across different clusters. This step significantly enhanced the reliability and interpretability of our cluster labels, thereby enriching our overall analysis.

4.3 Labeled Cluster after Merging

In the final phase of our analytical journey, we present a refined plot delineating labeled cellular clusters, achieved after judiciously merging clusters that represented identical cell types. Complementing this, the second and third plots capture the heterogeneity in cellular distribution across different patients, segregating them into control and treatment groups for meaningful comparison. Specifically, these groups encompass *Baseline Myocarditis cases*, *Baseline controls*, *Follow Up controls*, and *irAEs* (immune-related adverse events). The accompanying bar chart elucidates the proportional representation of each cell type within these patient groups. Notably, a distinctive divergence in cell type proportion is observed; the *Baseline controls* and *Follow Up controls* demonstrate a significantly higher presence of NKT cells, while *irAEs* and *Baseline Myocarditis cases* are characterized by an increased frequency of CD14+ Monocytes. These findings not only enrich our cellular landscape but also offer pivotal cues for further clinical and pathological evaluations.

References

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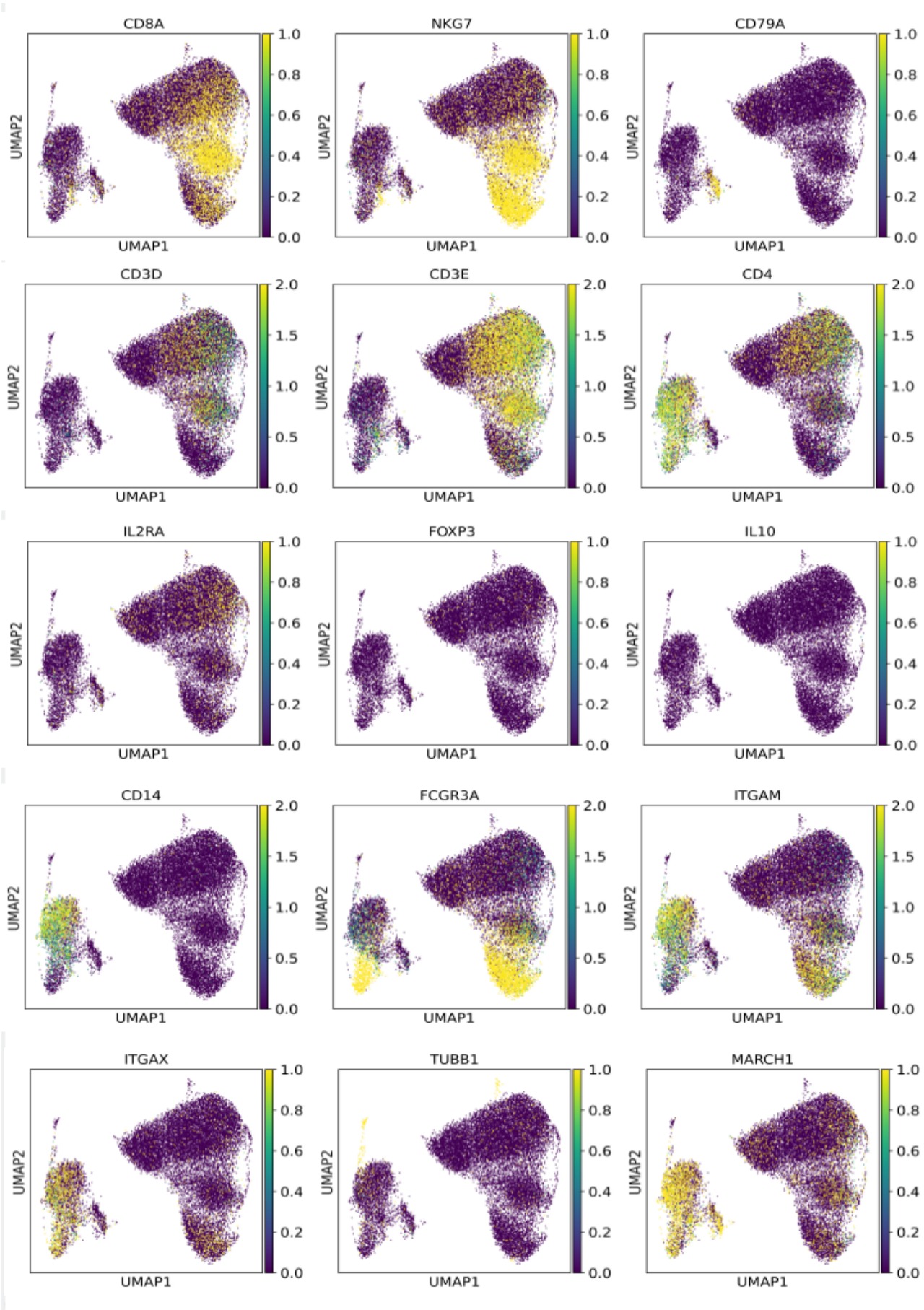

Figure 2: **marker genes visualization**

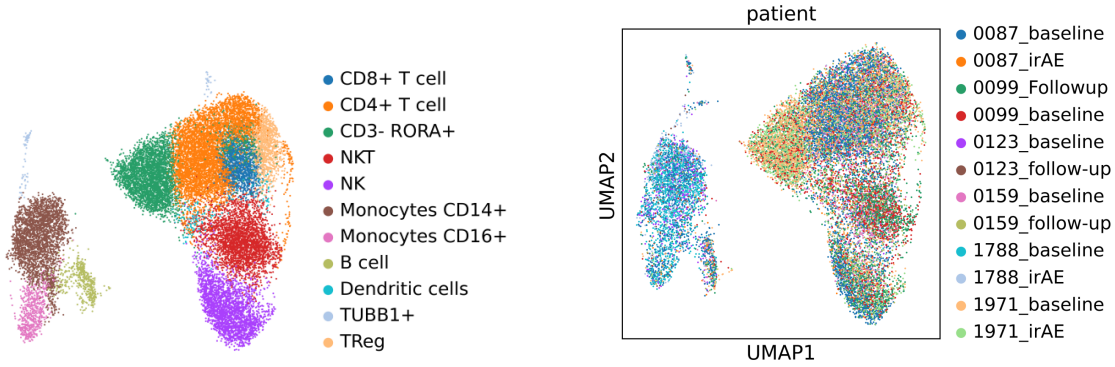

Figure 3: Distribution of Cell Type and Patient Category

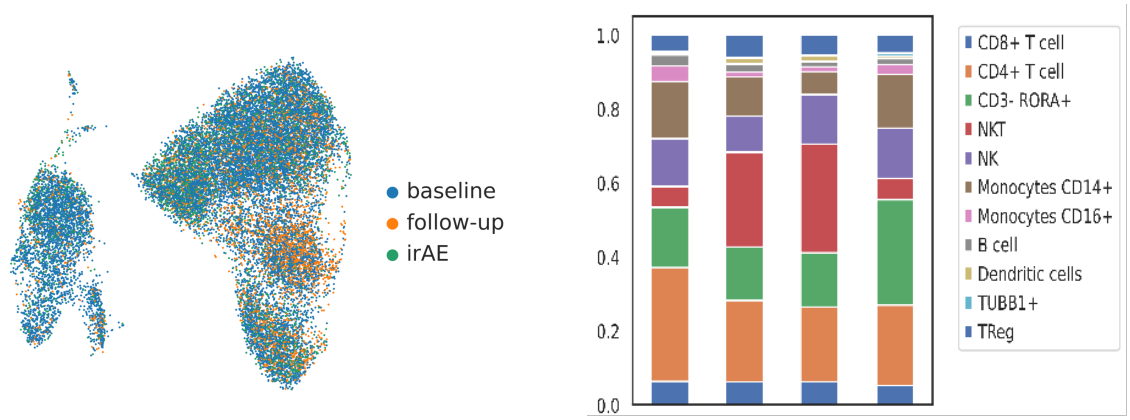

Figure 4: Distribution of Cell Type and Patient Category

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