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DoCTIS: A single cell RNA-Seq atlas of drug response to targeted therapies

Antonio Julià[17], Yolanda Guillén[1], Juan Angel Patiño Galindo[1], Sergio H. Martínez-Mateu[1], Paloma Vela Casasempere[2], Antonio Fernández Nebro[3], Carlos Marras[4], Santos Castañeda[5], Jaime Calvo Alén[6], Jesús Tornero Molina[7], Juan de Dios Cañete Crespillo[8], Eugeni Domènech[9], Javier Gisbert[5], Jose M Carrascosa[9], Eduardo Fonseca[10], Luis Bujanda Fernández de Pierola[11], Valle García Sánchez[12], Britta Siegmund[13], Giampiero Girolomoni[14], Holger Heyn[19], Laura Jiménez[19], Pere Santamaría[15], Edgar Angelats[8], Rick Myers[16], Ernest Choy[18], Sara Marsal[17]

[1]IMIDomics, Barcelona, Spain, [2]Hospital General Universitario de Alicante, Rheumatology, Alicante, Spain, [3]Hospital Regional Universitario Carlos Haya, Málaga, Spain, [4]Hospital Universitario Virgen de la Arrixaca, Rheumatology, Murcia, Spain, [5]Hospital Universitario La Princesa, Rheumatology, Madrid, Spain, [6]Hospital Universitario de Araba, Rheumatology, Vitoria, Spain, [7]Hospital Universitario de Guadalajara, Guadalajara, Spain, [8]Hospital Clínic-IDIBAPS, Barcelona, Spain, [9]Hospital Universitari Germans Trias i Pujol, Rheumatology, Badalona, Spain, [10]Complejo Hospitalario Universitario de A Coruña, A Coruña, Spain, [11]Hospital Universitario de Donostia, San Sebastián, Spain, [12]Hospital Universitario Reina Sofía, Rheumatology, Córdoba, Spain, [13]Charité- Universitätsmedizin Berlin, Berlin, Germany, [14]University of Verona, Verona, Italy, [15]Institut d'Investigacions Biomèdiques August Pi i Sunyer, Barcelona, Spain, [16]HudsonAlpha Institute for Biotechnology, Huntsville, United States of America, [17]Vall d'Hebron Hospital Research Institute, Rheumatology Research Group, Barcelona, Spain, [18]Cardiff University School of Medicine, Cardiff, United Kingdom, [19]Centro Nacional de Análisis Genómico (CNAG), Barcelona, Spain

Introduction

Targeted therapies have revolutionized the management of **immune-mediated inflammatory diseases (IMIDs)**. However, there is a substantial number of patients who respond poorly to a given drug. Understanding the mechanisms behind the response to drugs and patient heterogeneity would be of high utility. Such knowledge could be the basis of methods for the determination of the most suitable therapy for a given individudal.

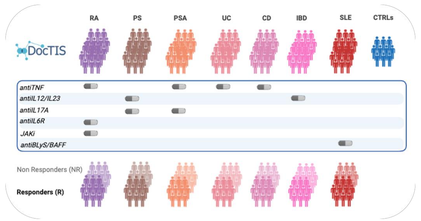
Objectives

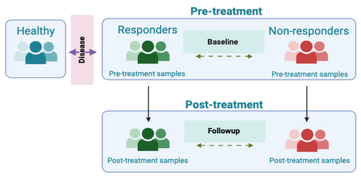
By integrating high-resolution transcriptomic data from eleven longitudinal cohorts representing six IMIDs: **rheumatoid arthritis (RA), psoriasis (Ps), psoriatic arthritis (PsA), Crohn's disease (CD), ulcerative colitis (UC), and systemic lupus erythematosus (SLE)**; and six therapies: **anti-IL17A, anti-TNF, anti-IL12/IL23, JAKi, anti-BLyS/BAFF, and antiIL-6R**; including **responders (R) and non-responders (NR)**, this study aims to:

- Identify shared and disease-specific signatures across autoimmune diseases.
- Characterize treatment-induced alterations in circulating immune cells.
- Explore molecular pathways associated with therapeutic response.

Methods

We applied **single cell RNA sequencing** to **360 PBMC samples** from **176 patients** from PS, PSA , CD, UC, RA, and SLE patients treated with six different drugs targeting TNF, IL12p40, IL6R, BLySS, IL17 and JAK pathways.



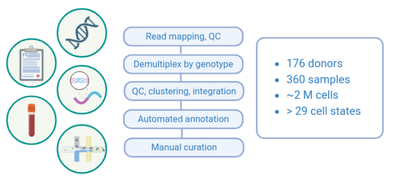


All patients were analyzed at **two time points** including baseline and at the week of clinical response. Blood samples were processed to generate PBMCs single cell RNA-Seq libraries.

Libraries were multiplexed using the **genotype** of each donor. Per each timepoint, NR and R samples were mixed in order to **minimize batch effect.** A minimum of 2,000 cells per condition were included.

Low quality cells were filtered out. Filtered cells were integrated, clustered, and labeled by combining automated and manually curated annotations.

The cellular composition, transcriptional configuration, and activity of different transcription factors were characterized across all conditions.



Results

Longitudinal atlas of drug response of 6 IMIDs to six targeted therapies

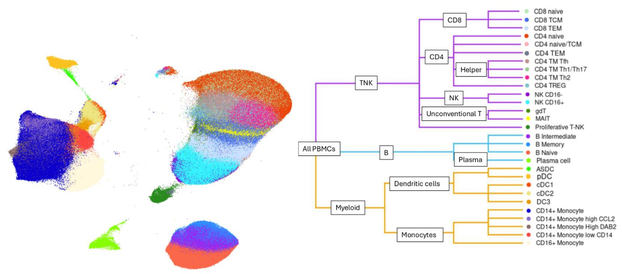


Figure 1: UMAP of scRNA-Seq data from all PBMCs.

PBMCs from 360 samples collected from 184 patients representing six IMIDs (RA, PS, PsA, CD, UC, and SLE) and 8 healthy controls, were profiled with scRNA-Seq (Fig. 1). A total of **2,096,581 single transcriptomes** were clustered into B, T/NK and myeloid lineages, leading to the identification of 29 cell types.

IMIDs differentiate by circulating cell abundance

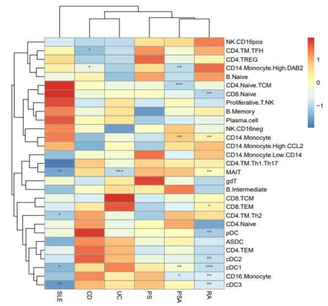


Figure 2. Significant differences in cell composition disease vs. Controls. *Scaled proportion of cell types.*

- All IMIDs but PS showed at least one immune cell population with a significant differentially represented cell population compared to controls (Fig. 2).
- RA displayed the largest systemic changes, marked by a general reduction of dendritic cells (DC) subtypes.
- Clustering by average cell abundance showed a higher similarity between the two IMIDs targeting the gut (CD and UC) and between the two arthritis (RA and PsA) than to the other diseases.
- SLE showed a compositional profile more differentiated from the rest of IMIDs.
- Circulating mucosa-associated invariant T cells (MAIT) were significantly reduced in RA, UC and SLE compared to controls.

IMIDs express distinct circulating expression and TFs programs

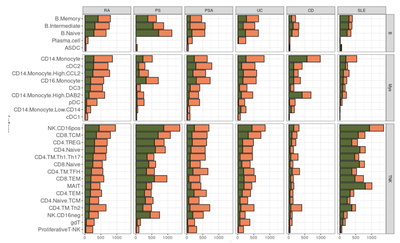


Figure 3. Differentially expressed genes disease vs control.

- We found large inter-disease differences at the genetic regulatory programs (Fig 3).
- Confirmation of a predominant interferon response signature affecting multiple cell types in SLE.
- Novel findings were reported, like the strong regulatory differences between disease with a shared genetic risk, like PSA and PS
- TF activity profile was found to have a cell-specific differential profile in IMIDs vs controls, especially in T and NK cells.

Impact of drugs on cell abundance is disease-specific

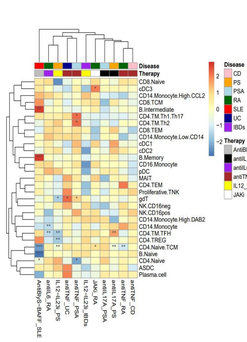


Figure 4. Significant differences in cell composition after therapy.

- The impact of each drug was found to be largely disease-specific (Fig. 4).
- AntiBlyS in SLE showed the broadest effect on cell composition, affecting mostly the B compartment.
- In RA, antiTNF reduces the frequency of CD4 T naive/CM cells, whilst in PsA it increases the abundance of Th1/Th17 CM and Th2 CM cells and reduces the abundance of CD4 naive T cells.

Response is associated to differential expression of drug targets

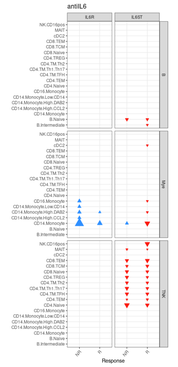


Figure 5. Differentially expressed drug target genes after antiIL6R therapy in RA between R and NR.

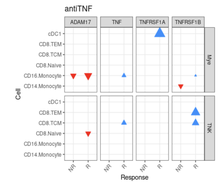


Figure 6. Differentially expressed drug target genes after antiTNF therapy in RA between R and NR.

- Most transcriptional changes affecting IL6R in antiIL6R treated RA patients (Fig. 5), occur in monocytes.
- RA AntiIL6R NR and R show a distinct pattern of IL6ST expression in classical monocytes after therapy, a key element in IL6 pathway regulation.
- In RA, antiTNF Responders showed an upregulation of key targeted elements such as TNF receptors in myeloid, and CD8 T cells (Fig. 6).

Conclusions

The scRNA-Seq atlas developed in DoCTIS provides a **unique resource** for the understanding of **drug response and disease heterogeneity** across immune-mediated inflammatory diseases.

- Cell composition and cell-specific transcriptional configuration of circulating PBMCs is IMID-dependent.
- Therapy leads to disease-specific changes in circulating cell composition and gene expression. Response is associated to the abundance of difference cell populations and changes in the expression of targeted genes
- Responders and non-responders showed differences in drug target expression after therapy.
- Further analyses will shed light on cell specific alterations associated to therapy response, before and after treatment.

Disclosures: S. Castañeda: Bristol-Myers Squibb(BMS), Eli Lilly, Merck/MSD, Pfizer, Roche, UCB; **E. Choy**: AbbVie, Amgen, Bio-Cancer, Biocon, Biogen, Bristol-Myers Squibb(BMS), Chugai, Eli Lilly, Fresenus Kabi, Galapagos, Gilead, Janssen, Novartis, Pfizer, Regeneron, Roche, RPharm, Sanofi, UCB Pharma; **H. Heyn**: Mirxes, Moderna, Nanostring, Omniscope, Singularity; **Sara Marsal** and **Toni Julià:** IMIDomics; The remaining authors declare no conflicts of interest

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