# Characterization of the Immune-Driven Drug Hypersensitivity by Single-cell RNA Sequencing Technologies

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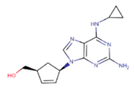
## Adverse Drug Reaction

Adverse drug reactions (ADRs) remain as a major burden to the patients and healthcare system. Among all, Immune-mediated ADRs (IM-ADRs) comprise approximately 20% of all, and often lead to life-threatening symptoms as such, Stevens-Johnson syndrome (SJS)/Toxic epidermal necrolysis (TEN), and severe cutaneous adverse reactions (SCARs). Increasing evidences in IM-ADRs report strong association with certain class I or II human leukocyte antigen (HLA), and involvement of drug-reactive T lymphocytes

## Abacavir (ABC) Hypersensitivity

A nucleoside analog reverse transcriptase inhibitor (NRTI) used to treat HIV and AIDS

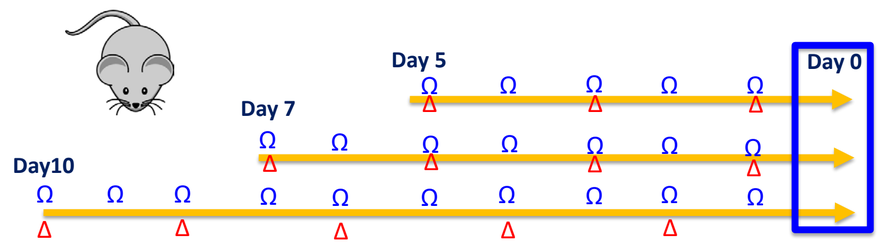


A synthetic carbocyclic nucleoside

The hypersensitivity reactions occur within the first 4-6 weeks of therapy and are reversible with discontinuation of use

FDA recommends the screening of the patients for HLA-B57:01 prior to initiating treatment with ABC (FDA Alert on July 24th, 2008 )

## Study Design

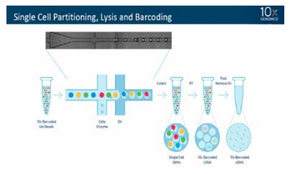



Ω 3mg of ABC i.p. injection every day

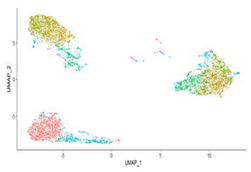
Δ 0.2 µg of ABC ear painting every other day

## Single Cell RNA Sequencing

**Single cell sequencing** surveys the genetic information from individual cells with NGS technologies. It provides a higher resolution of cellular differences and a deeper insight on the functionality of an individual cell in the microenvironment




10x Genomics based Droplet-based single cell RNA seq technologies



Microenvironment of mouse lymph nodes

## Goal of the Study

**Two goals:**

**-Assessment of Hypersensitivity Inducing PD1⁺CD8+ T cells**

**-Mapping microenvironment of mouse lymph nodes**

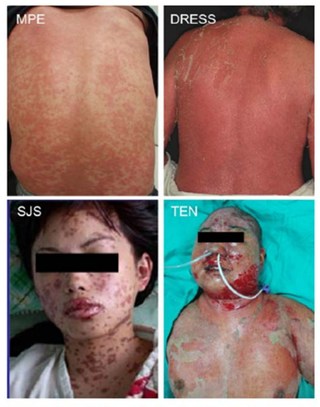
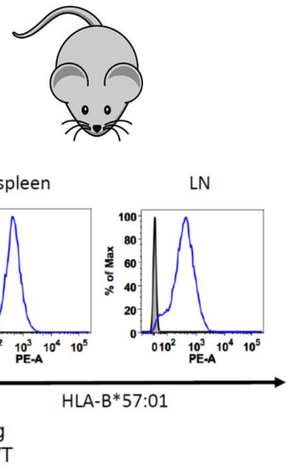



**Figure 1.** Clinical presentations of cutaneous adverse drug reactions. DRESS, drug rash with eosinophilia and systemic symptoms; MPE, maculopapular exanthema; SJS, Stevens–Johnson syndrome; TEN, toxic epidermal necrolysis.

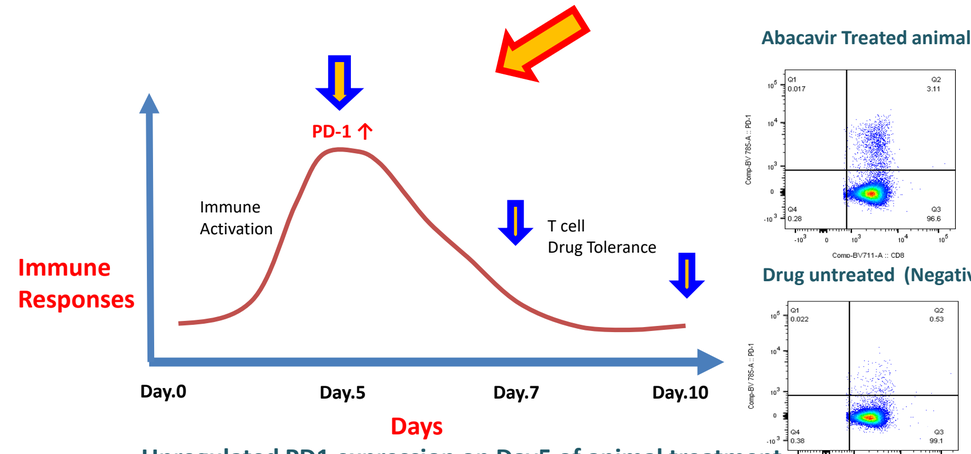
Clinical Manifestation of ADRs

Sever cutaneous adverse drug reactions: 2016




HLA-B*57:01 expressing transgenic mouse model

Cardone, et al. JCI 2018




Upregulated PD1 expression on Day5 of animal treatment

Adapted from Cardone et al: JCI 2018

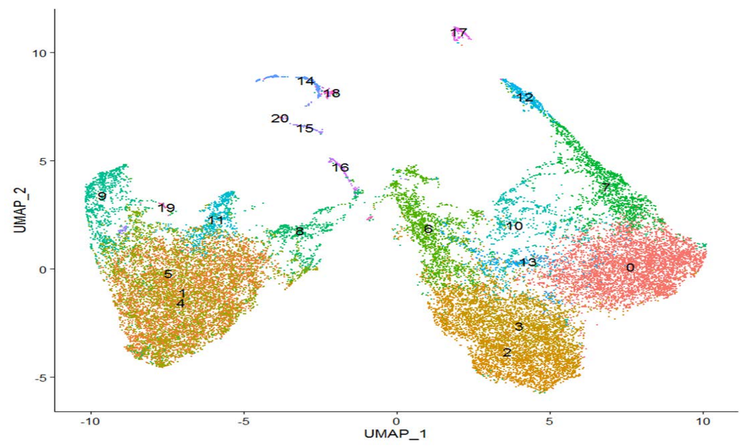
Abacavir Treated animals




Drug untreated (Negative Ct)




## Single Cell Transcriptional Profiling of Mouse Lymph Nodes



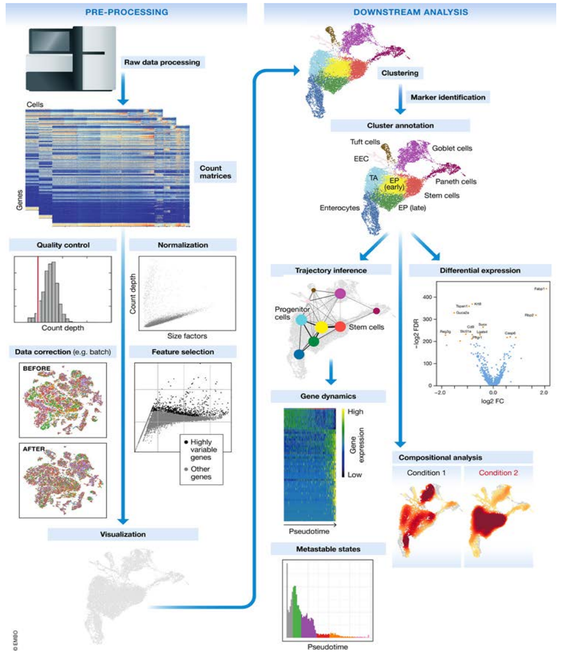

Landscape of mouse lymph nodes

In this study, we used single cell RNA sequencing technologies to evaluate immune cell responses in the lymph nodes during the initial stages of in vivo drug treatment in our HLA transgenic mouse.

We collected the transcriptome data of 32,000 single cells from the animals at four different time points of drug exposures and evaluated the differentially expressed high-resolution transcriptomes. Out data revealed that 1) a complex cellular environment in mouse lymph nodes, 2) a heterogeneous effect of drug administration on T cell and antigen presenting cell populations involved in drug specific reactions in each time points, and 3) the gene profiles of minor cell populations that represents drug-activated CD8+ T cells.

This work provides a frame-work for understanding drug induced IM-ADRs at the single cell level and a foundation for applying next generation sequencing technology to ensure drug safety.

## Analysis pipeline




Schematic of a typical single-cell RNA-seq analysis flow

Luecken et al, 2019 Molecular system biology

## Disclaimer

**The ideas, findings, and conclusions in this presentation have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any Agency determination or policy.**