Single cell RNA-sequencing reveals the transcriptional impact of epigenetic inhibitors #4351

Sungmi Park, Barbara M. Bryant, Michael Steinbaugh, Archana Bommi-Reddy, and Andrew R. Conery

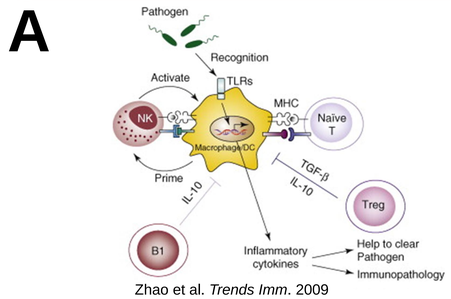
Constellation PHARMACEUTICALS

ABSTRACT

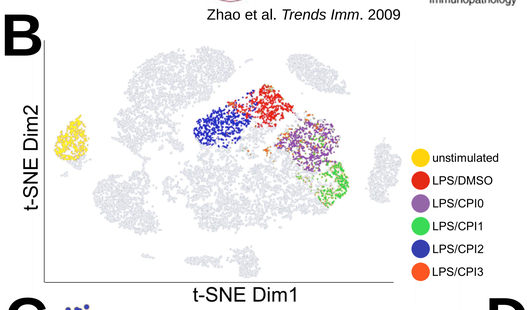
Transcriptional reprogramming and plasticity are critical for dynamic events at the organism and tissue level. Activation of innate immune cells in both normal and pathogenic conditions results from the induction of transcriptional programs that polarize naïve monocytic cells toward specialized cell types that resolve infection and promote adaptive immunity. Tumor formation and evolution require adaptation to strong selective pressures, for which transcriptional plasticity is essential. Small molecule inhibition of the chromatin factors that regulate pathogenic transcriptional states may serve as a therapeutic option for inflammatory diseases and cancers. A central challenge in the development of epigenetic therapy is the ability to monitor transcriptional impacts in dynamic and heterogeneous cell populations.

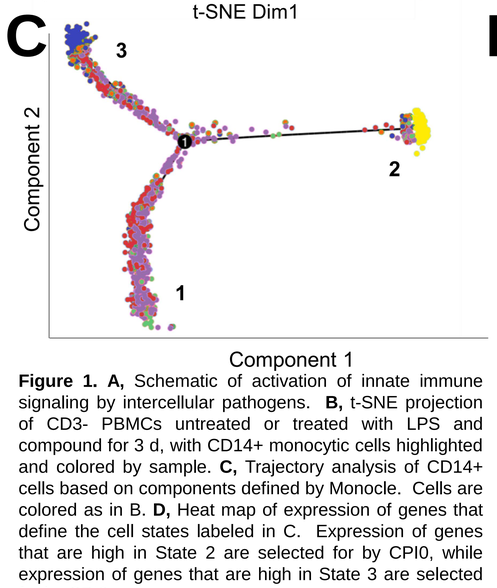
To explore this issue, we used single cell RNA-sequencing to characterize the transcriptional response of human cancer and primary cell populations to multiple inhibitors representing distinct target classes. We first show that *in vitro* activation of primary immune cells in a mixed population is skewed in a compound-specific manner. Next, we show in a human breast cancer cell line that inhibition with a novel CBP/EP300 catalytic inhibitor has distinct transcriptional effects from a direct estrogen receptor inhibitor, in part through enrichment of specific pre-existing transcriptional states.

Overall, our results demonstrate the value of single cell transcriptomics for understanding the response of heterogeneous cell populations to epigenetic inhibitors and for enhancing the therapeutic potential of targeting pathogenic transcriptional programs.



Zhao et al. *Trends Imm*. 2009





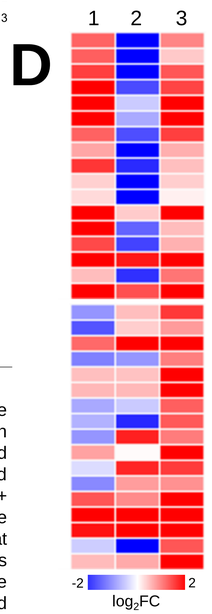
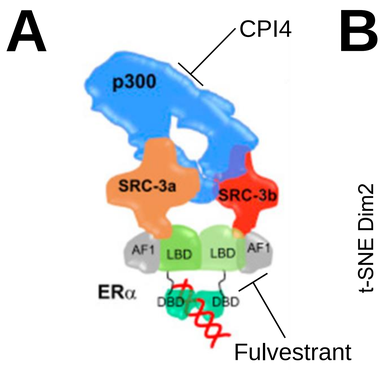
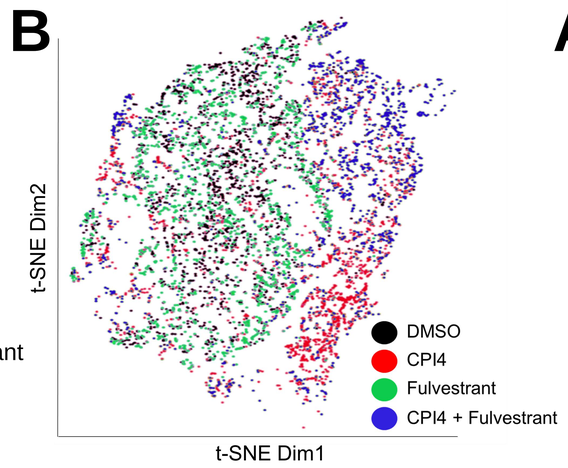
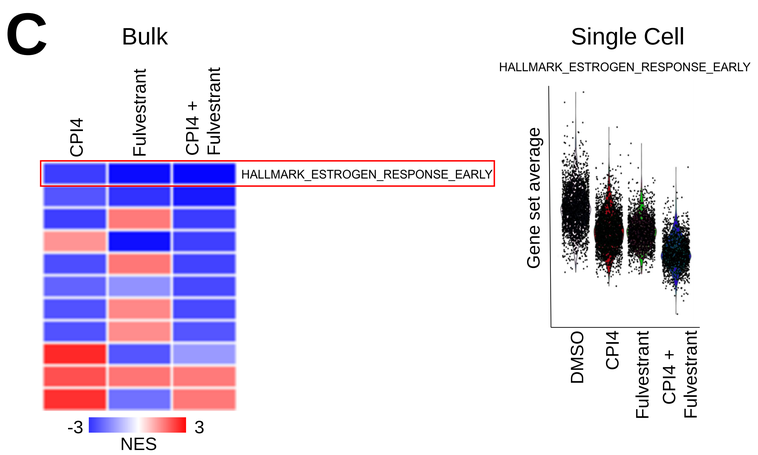


Figure 1. A, Schematic of activation of innate immune signaling by intercellular pathogens. **B,** t-SNE projection of CD3- PBMCs untreated or treated with LPS and compound for 3 d, with CD14+ monocytic cells highlighted and colored by sample. **C,** Trajectory analysis of CD14+ cells based on components defined by Monocle. Cells are colored as in B. **D,** Heat map of expression of genes that define the cell states labeled in C. Expression of genes that are high in State 2 are selected for by CPI0, while expression of genes that are high in State 3 are selected for by CPI2.



Yi et al., *Mol. Cell*. 2015





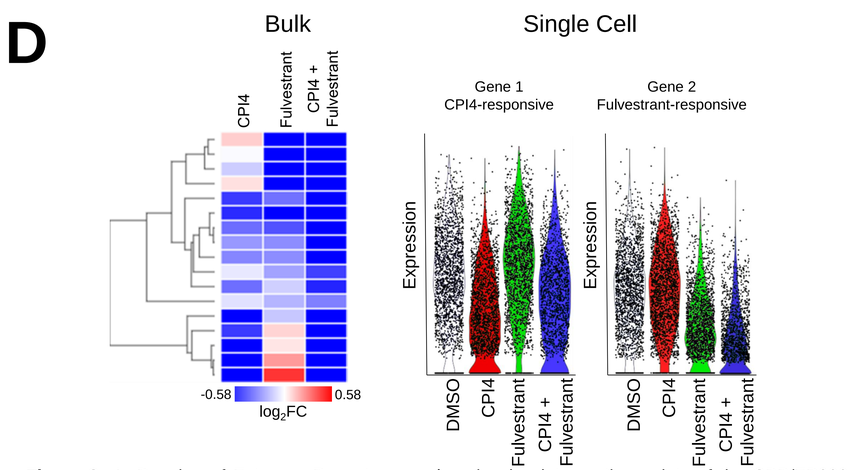
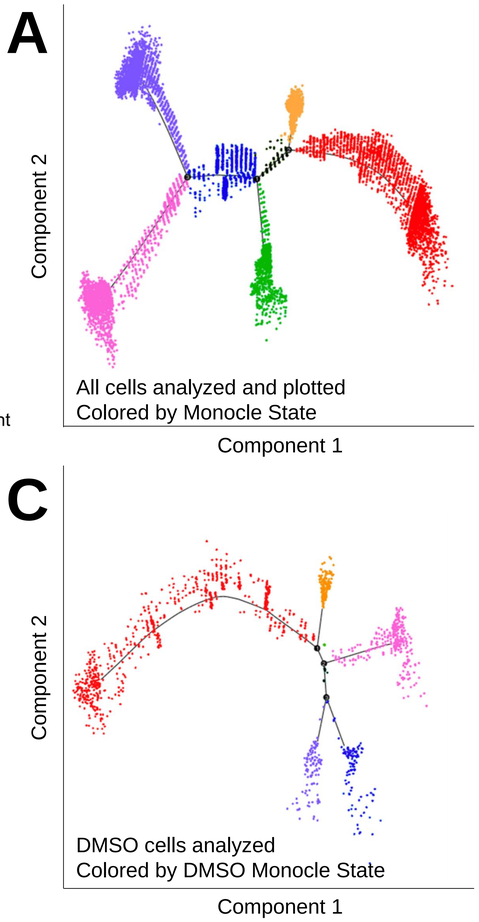
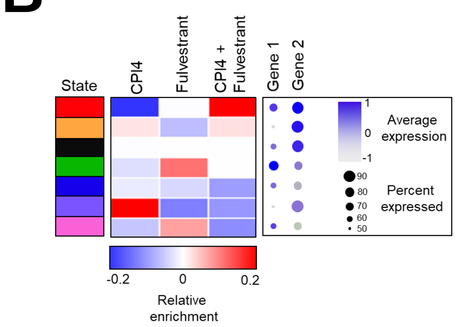
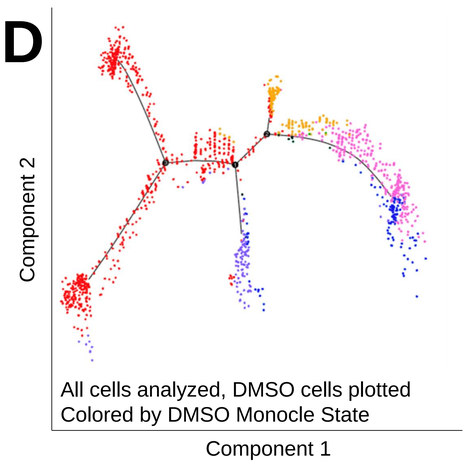


Figure 2. A, Drawing of Estrogen Receptor complex showing intervention points of the CBP/EP300 catalytic inhibitor CPI4 and the SERD Fulvestrant. **B,** t-SNE projection of MCF-7 cells treated with the indicated compounds for 6 hr. Single cell RNA-seq libraries were prepared using the 10X Genomics Chromium instrument according to manufacturer's instructions. Sequencing reads were deconvoluted and mapped to hg38 using Cell Ranger version 3.0. Dimensionality reduction, clustering, and differential expression were carried out using Seurat. **C** (left)**,** Gene set enrichment analysis of bulk RNA-seq data for treatment of MCF-7 cells as in B showing NES of top enriched signatures in the Hallmark gene signatures list (MSigDB). (right) GSEA of HALLMARK_ESTROGEN_RESPONSE_EARLY geneset in single cell RNA-seq. Expression of all genes in the gene set was averaged for each cell and plotted according to sample treatment. **D,** Expression of selected genes in the Hallmark_Estrogen_Response_Early gene set in bulk (left) and single cell (right) RNA-seq data. For single cell RNA-seq, expression of two example genes from the heatmap was determined for each cell and plotted according to sample treatment.







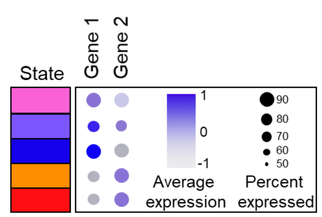


Figure 3. A, Monocle trajectory of cells described in Figure 2B. Cells are colored by State as defined by Monocle. **B,** (left) Fraction of cells within each State was calculated for each sample and DMSO fraction was subtracted to determine relative enrichment. (right) Average expression (normalized counts) for cells in each state was determined. Color scale shows average expression and size of circles shows the percentage of cells with detected counts. **C,** Trajectory analysis of only DMSO treated cells was carried out using Monocle. Cells are colored by State. **D,** Same trajectory as in A, but with only DMSO cells shown. Cells are colored by State as defined in C. **E**, Average gene expression of cells in each State for trajectory described in **C**. Two states with low cell numbers are not shown. Expression values are as described in B.

CONCLUSIONS

- Activation of primary monocytes by LPS in the presence of epigenetic inhibitors targeting distinct factors results in divergent transcriptional polarization, and may lead to new therapeutic hypotheses for the treatment of inflammatory disease.
- Both bulk and single cell RNA-sequencing of MCF-7 cells demonstrate that the CBP/EP300 inhibitor CPI4 targets ER transcriptional signaling in an orthogonal manner to the SERD Fulvestrant.
- Trajectory analysis supports the existence of pre-existing transcriptional states that may be enriched by small molecule inhibition.
- Ongoing characterization of transcriptionally sensitive transcriptional states may lead to biomarkers for therapeutic intervention with CBP/EP300 inhibitors.