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CANCER CENTER

# ***Transcriptional dissection reveals antitumor role of T follicular helper cells in head and neck cancer***

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## **Mentors**

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*November 8, 2019*

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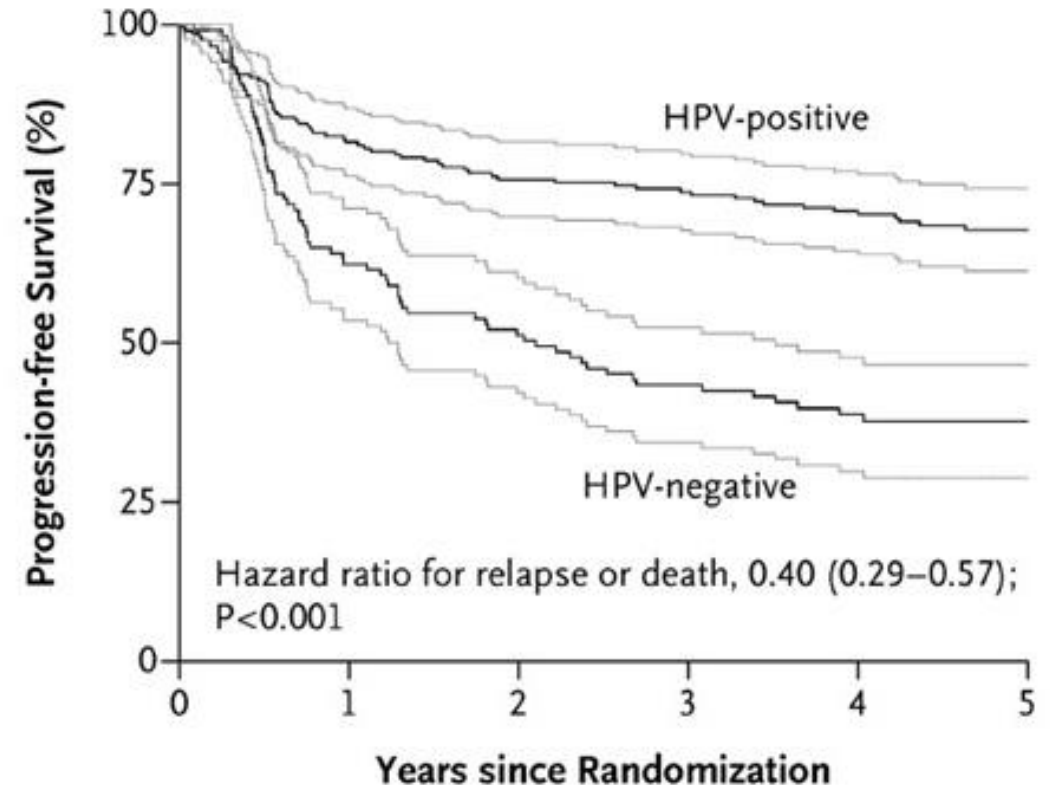
# Etiology and clinical characteristics of head and neck cancer

Ang et al, NEJM 2010

## ***Carcinogen-induced***

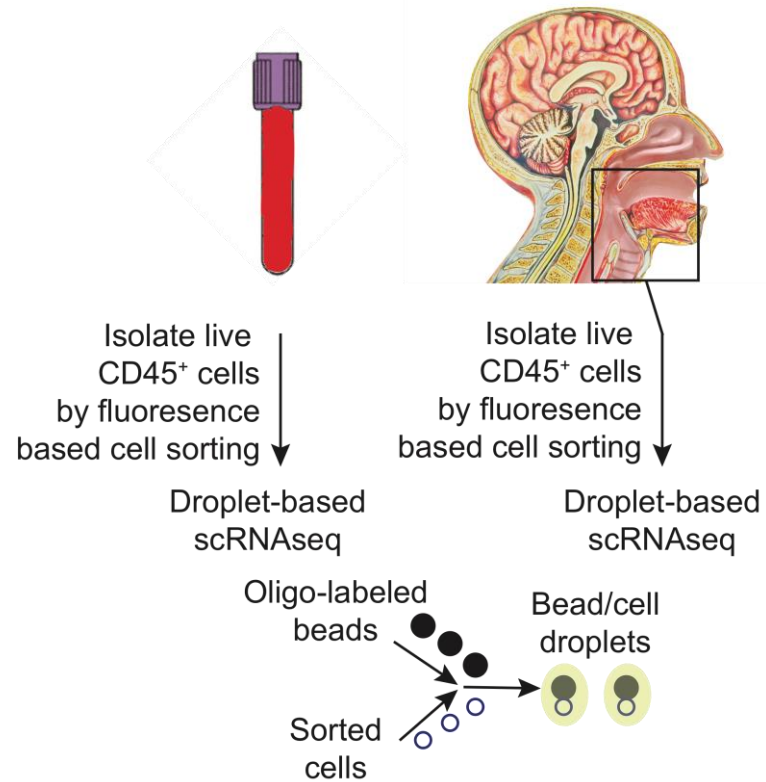


## ***HPV-induced***



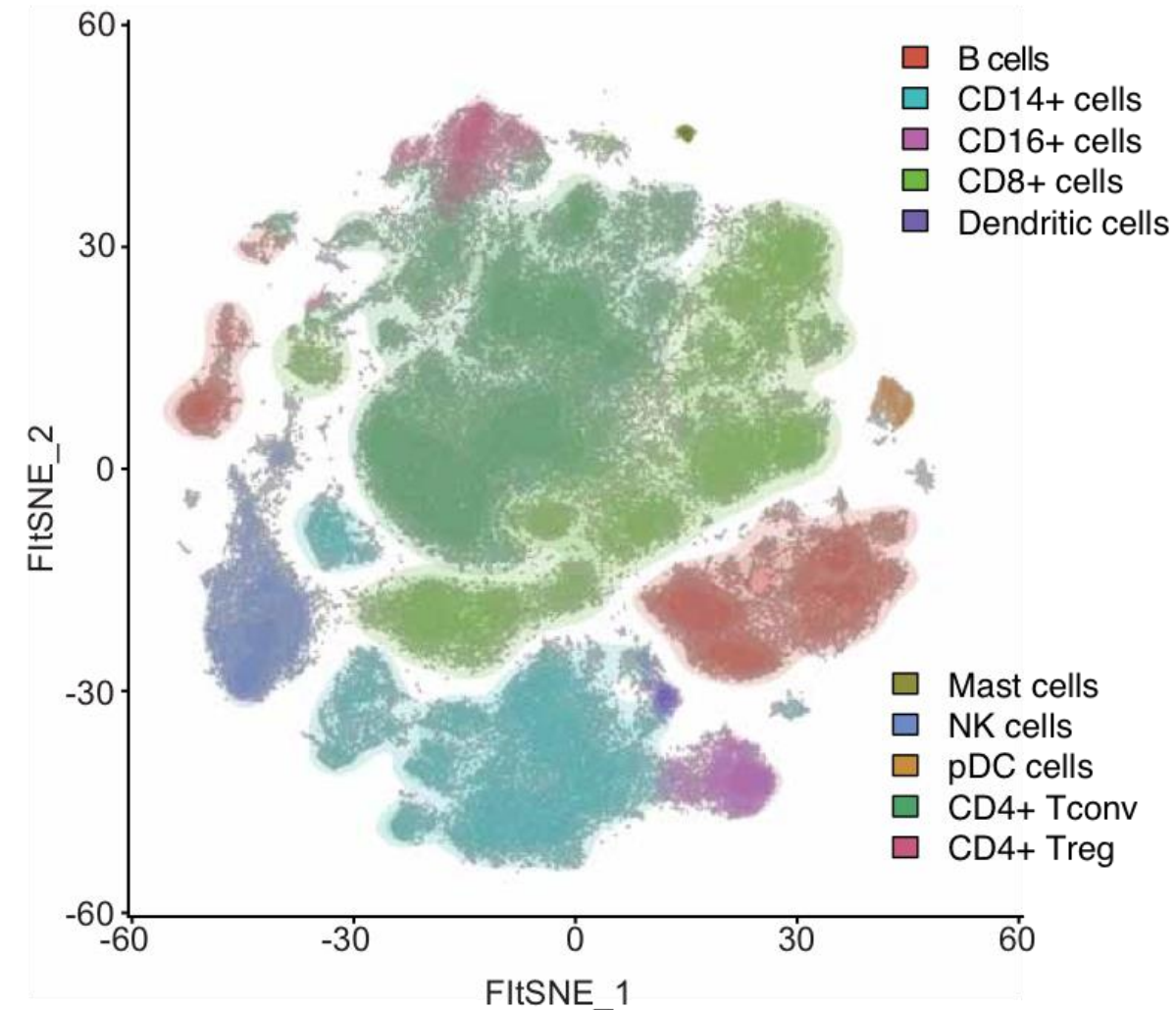
***We hypothesize viral- and carcinogen-induced HNSCC are associated with differences in antitumor immunity***

# Patient cohort and identification of major immune lineages

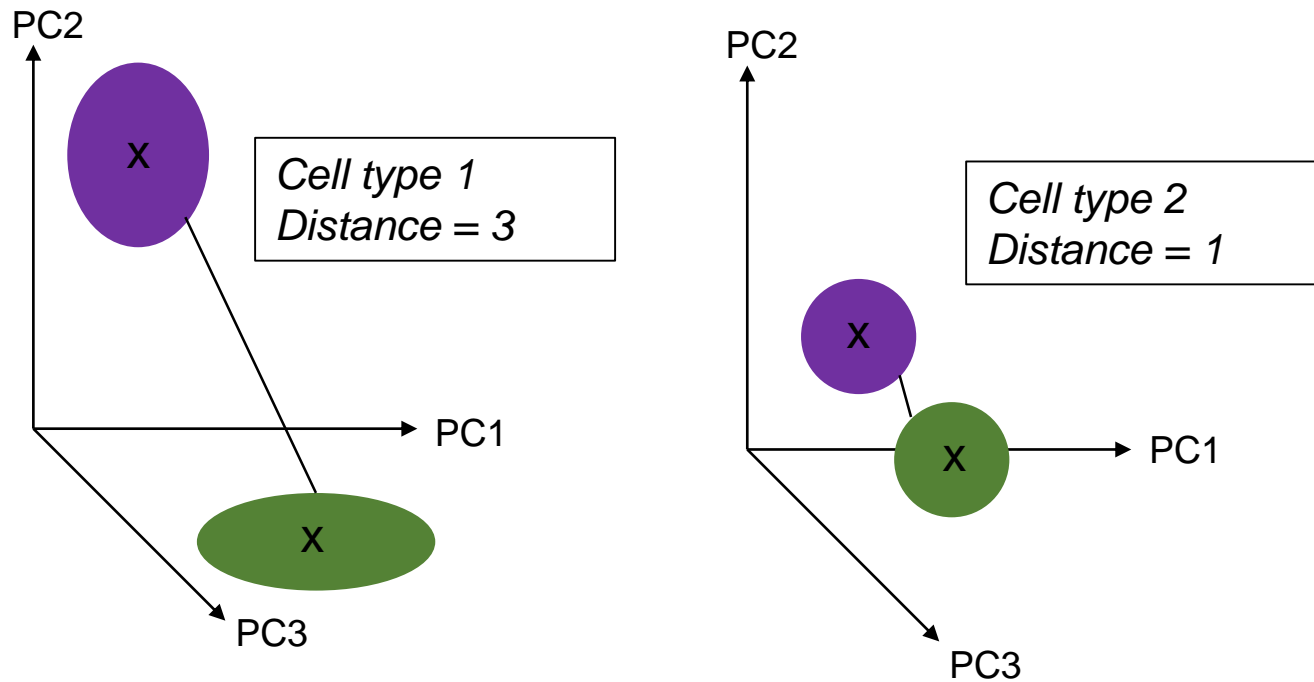


- Paired blood and tumor from 18 HPV- and 8 HPV<sup>+</sup> patients
- Blood from 6 healthy donors
- Tonsil tissue from 5 sleep apnea patients

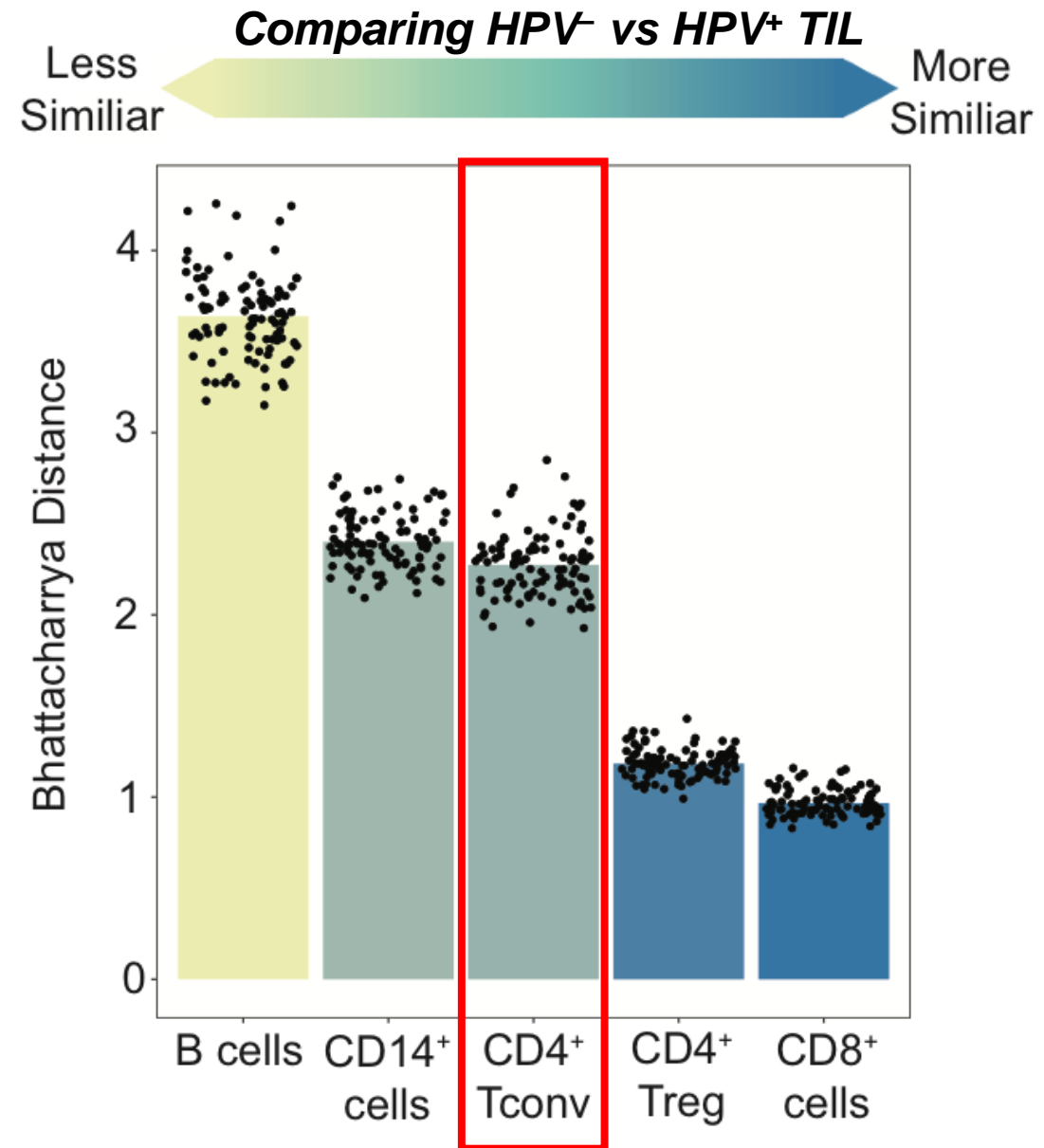
**131,224 single cells analyzed**



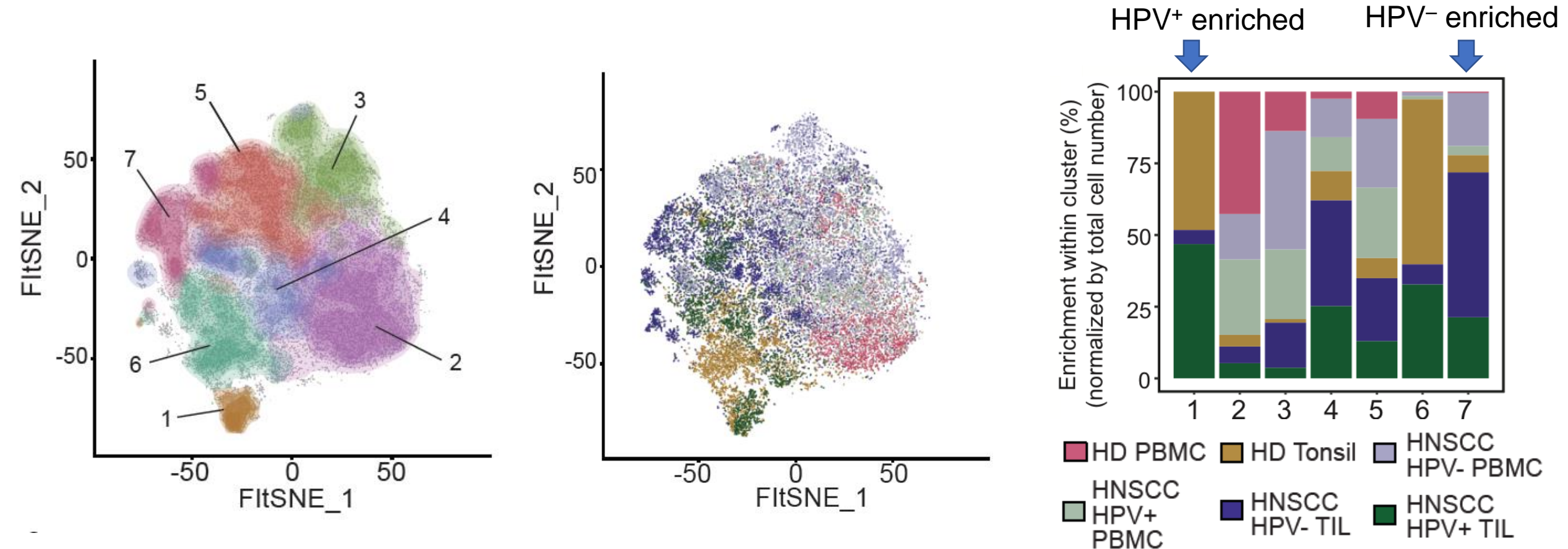
# Quantifying differences in immune lineages between HPV- and HPV+ HNSCC



- Distance between distributions is known as the Bhattacharyya Distance (BD)
- Greater BD = greater difference between populations

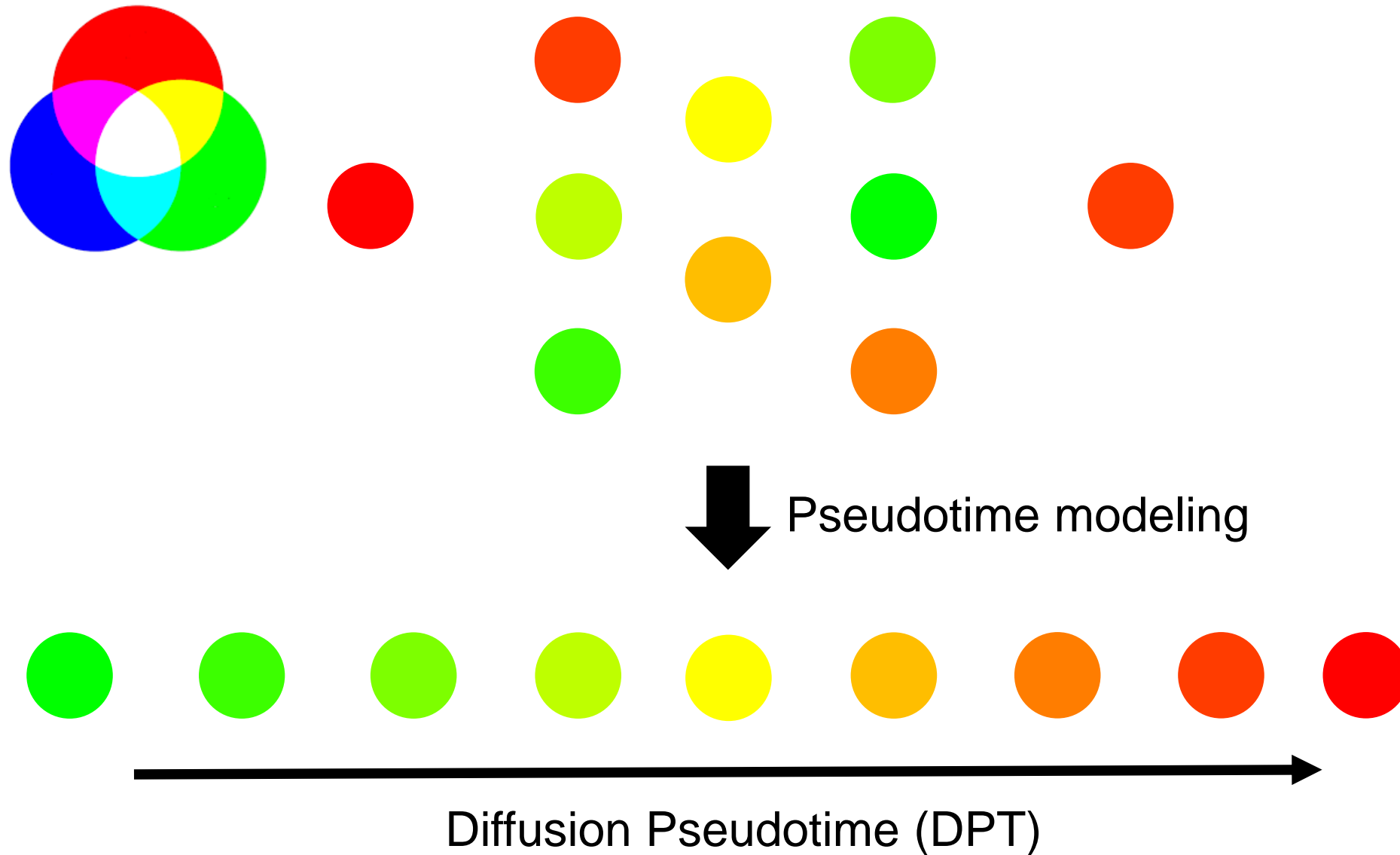


# Dissection of CD4<sup>+</sup> T<sub>conv</sub> in HPV<sup>-</sup> vs HPV<sup>+</sup> HNSCC



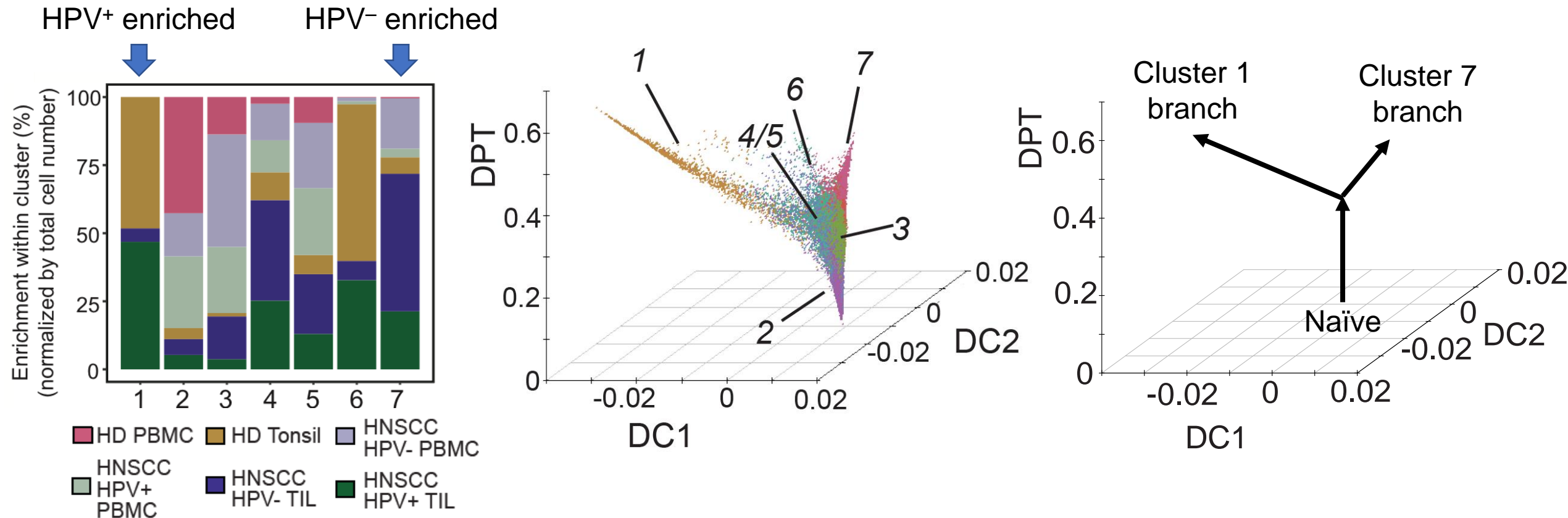
- Identified 41,889 CD4<sup>+</sup> T<sub>conv</sub> from all samples
- CD4<sup>+</sup> T<sub>conv</sub> from HPV<sup>+</sup> TIL were enriched in cluster 1, while CD4<sup>+</sup> T<sub>conv</sub> from HPV<sup>-</sup> TIL were enriched in cluster 7

# Diffusion analysis provides insight into differentiation trajectories



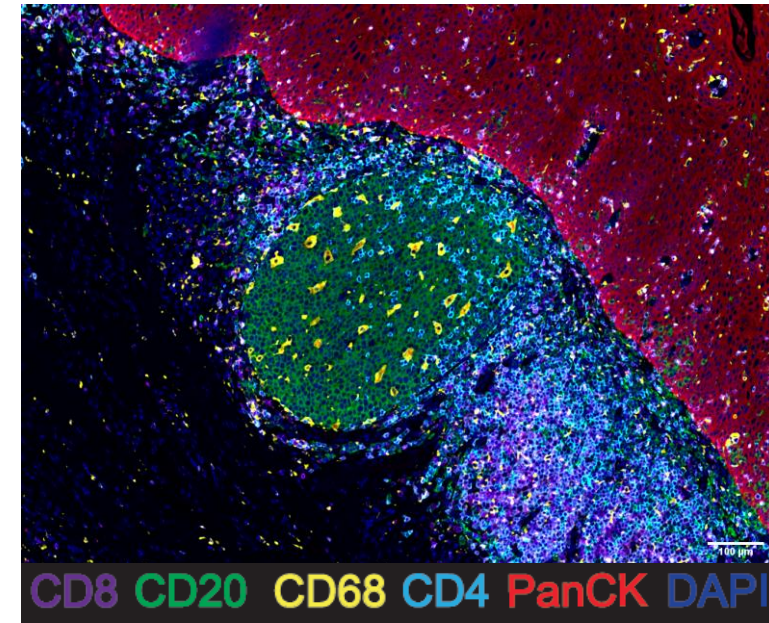
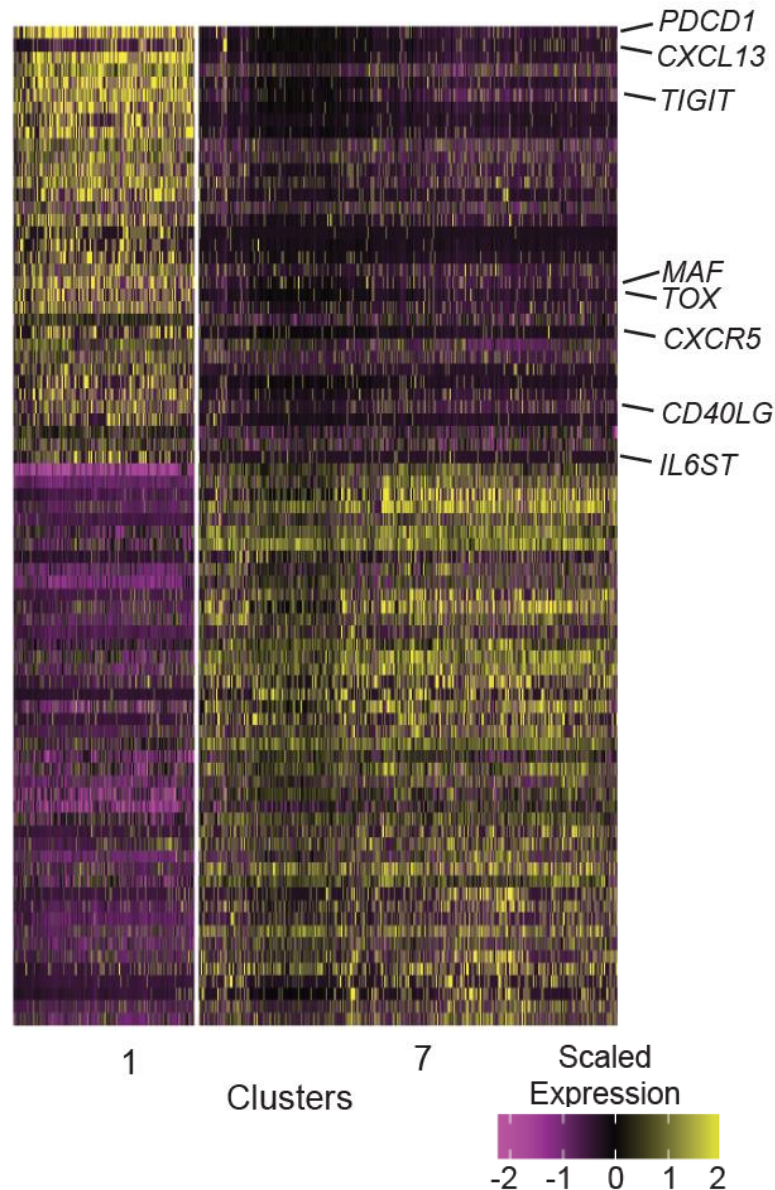
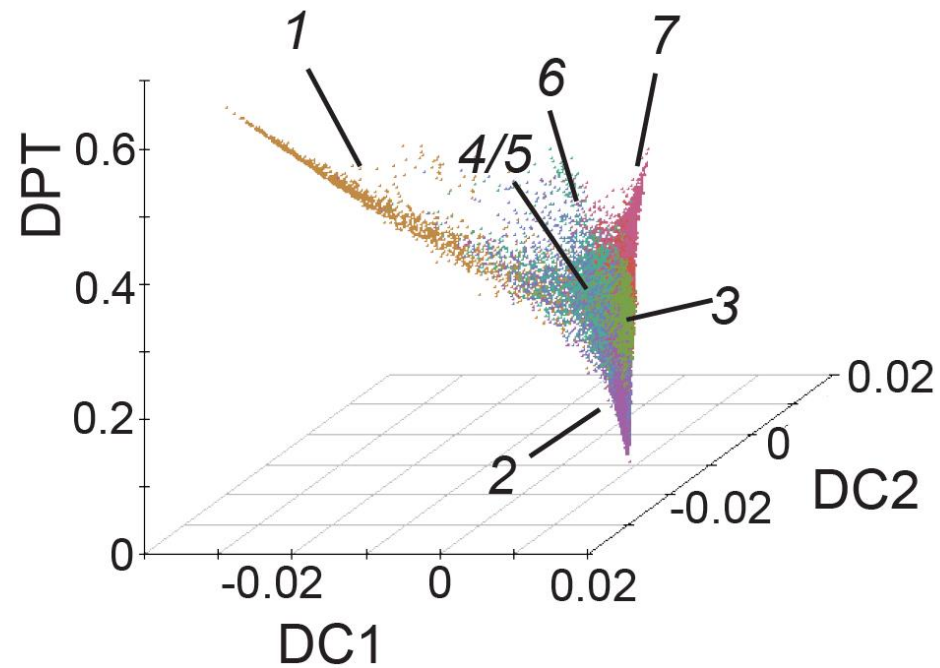


# CD4<sup>+</sup> T<sub>conv</sub> exhibit divergent differentiation trajectories



- Differentiation begins with naïve CD4<sup>+</sup> T cells becoming activated and progressing through early activation
- Trajectories then branch, with the Cluster 1 branch associated with HPV<sup>+</sup> TIL, while the Cluster 7 branch is associated with HPV<sup>-</sup> TIL

# CD4<sup>+</sup> T<sub>FH</sub> signature genes expressed in cluster 1



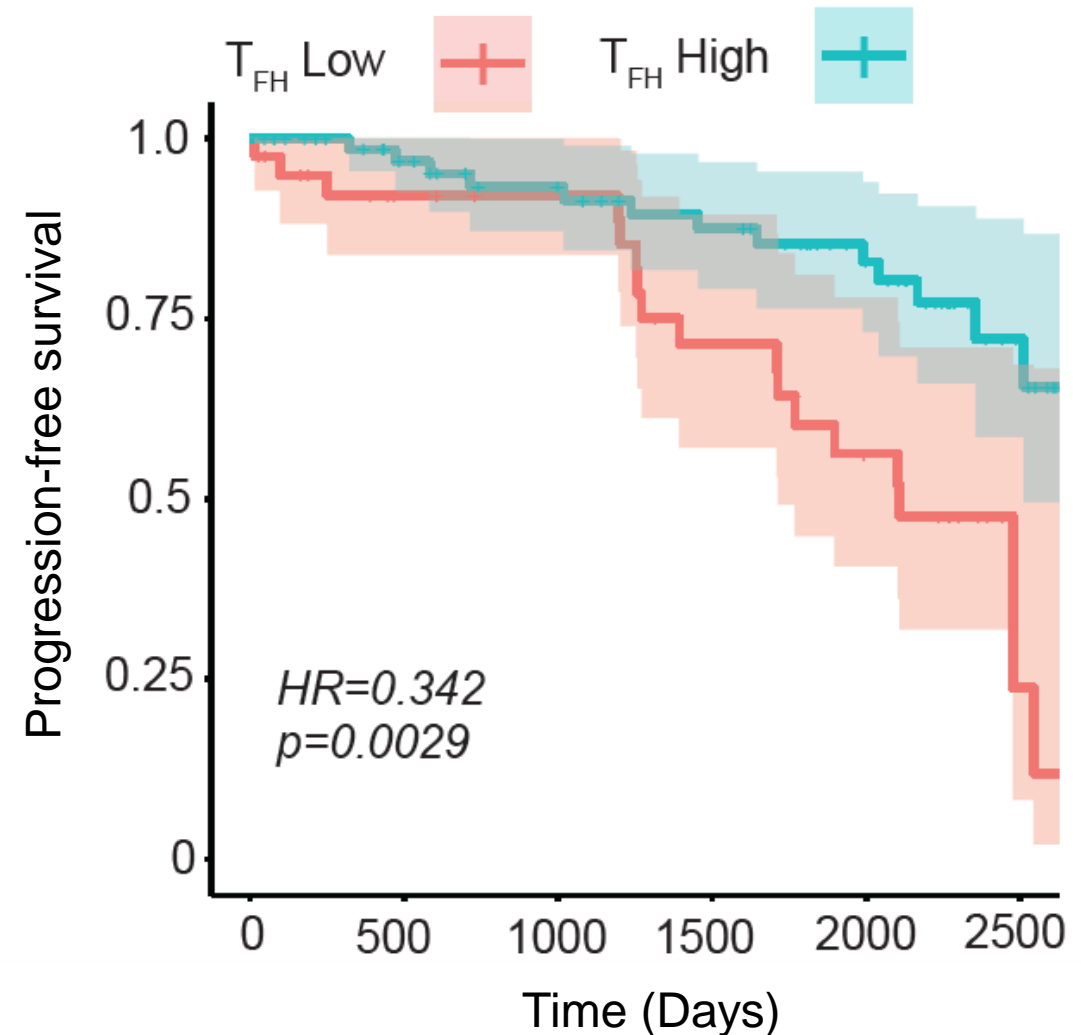
- Performed differential expression analysis, comparing clusters 1 and 7
- CD4<sup>+</sup> T follicular helper (T<sub>FH</sub>) genes are upregulated in cluster 1

- Immunofluorescence (7-color) analysis revealed tertiary lymphoid structures, supporting the presence of functional CD4<sup>+</sup> T<sub>FH</sub>

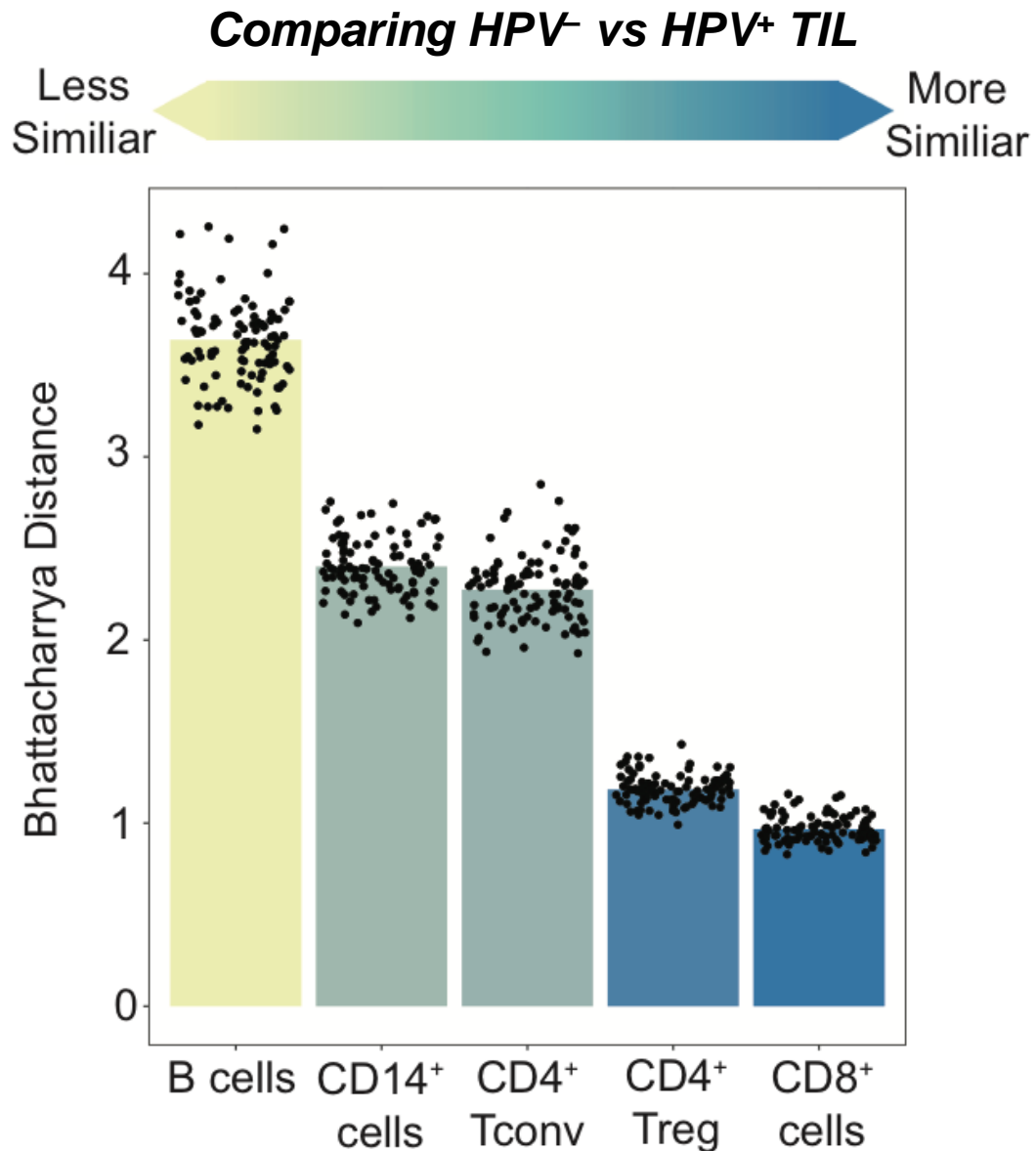


# $T_{FH}$ enrichment is associated with better progression free survival

- Utilized clinical data and bulk mRNAseq data from The Cancer Genome Atlas
  - 111 HNSCC patients with bulk mRNAseq, progression free survival data, and confirmed HPV status was used for survival analysis
- Determined a  $T_{FH}$  gene set enrichment score for each patient
- Stratified patients by high and low  $T_{FH}$  signatures



# scRNAseq reveals spectrum of differences in immune lineages between viral- and carcinogen-induced HNSCC



- A spectrum of differences exists between immune lineages in viral- versus carcinogen-induced HNSCC
  - CD8<sup>+</sup> T cells and CD4<sup>+</sup> T<sub>reg</sub> are relatively similar in both types of HNSCC
  - Germinal center B cells, CD4<sup>+</sup> T<sub>FH</sub> present in HPV<sup>+</sup> HNSCC
  - Presence of viral antigens during carcinogenesis may potentiate humoral arm of adaptive immunity
- Design of optimal immunotherapy should be predicated on the specific features of each immune lineage within the tumor microenvironment

# Acknowledgements

## **Bruno/Vignali Lab**

- Dario Vignali
- Tullia Bruno
- Sayali Onkar
- Lawrence Andrews
- Erin Brunazzi
- Carly Cardello
- Chris Chuckran
- Becky Dadey
- Angela Gocher
- Sheryl Kunning
- Caleb Lampenfeld
- Gracie Liu
- Mia Liu
- Ayana Ruffin
- Elisa Ruffo
- Feng Shan
- Ashwin Somasundaram
- Kate Vignali
- Haiguang Wang
- Creg Workman
- Richard Wu
- Hiroshi Yano
- Chen Ye

## **Chen Lab**

- Wei Chen
- Ting Wang



## **Vignali Lab (former)**

- Irina Abecassis
- Subinoy Biswas
- Maggie Liao
- Jessie Moskovitz
- Abby Overacre-Delgoffe
- Sherry Zhang

## **Pitt Sequencing Core**

- William Horne

## **Ferris Lab**

- Robert Ferris
- Cornelius Kurten
- Aditi Kulkarni

## **Hillman Flow Core**

- Mike Meyer
- Bratislav Janjic

## **Lafyatis Lab**

- Robert Lafyatis
- Tracy Tabib
- Zengbiao Qi

## **Pitt IMCPL**

- Jason Devlin

## **Patients and their families**



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**PittCRC**

Center for Research Computing

## **Funding Sources**

Cancer Immunology  
Training Program (T32)

Hillman Post-doctoral  
Fellowship Program

## **HNSCC SPORE**

## **University of Colorado**

- Kimberly Jordan
- Angela Minic