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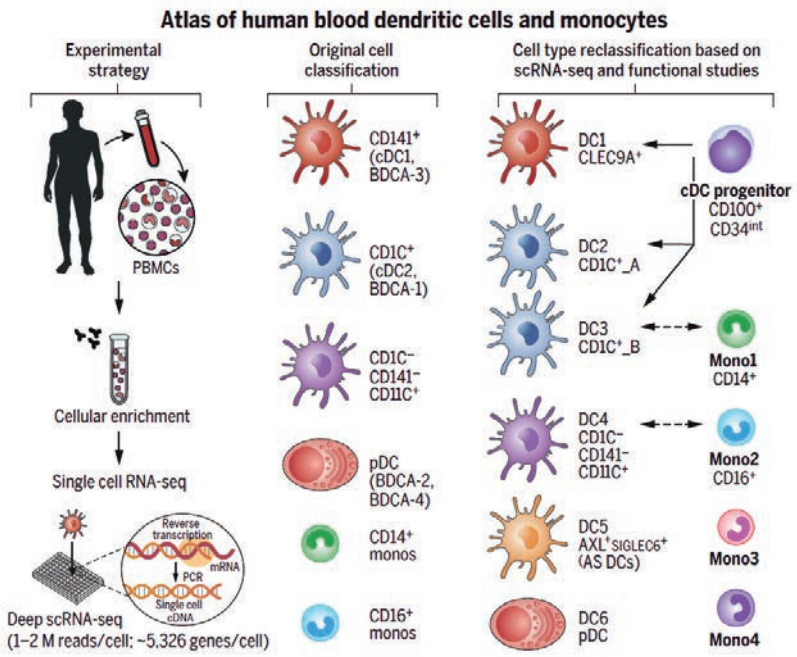
The Villani laboratory seeks to establish a comprehensive roadmap of the human immune system by achieving a higher resolution definition and functional characterization of cell subsets and rules governing immune response regulation, as a foundation to decipher how immunity is dysregulated in diseases. We use unbiased systems immunology approaches, cutting-edge immunogenomics, single-cell 'multi-omics' strategies, and integrative computational frameworks to empower the study and modeling of the immune system as a function of "healthy" and inflammatory states, disease progression, and response to treatment. Our multi-disciplinary team of immunologists, geneticist, computational biologists, and physicians work towards answering several key questions: Do we know all existing blood immune cell subsets? How do circulating immune cells mirror those in tissue microenvironment in the context of health and disease? Can we identify targets that would improve immunotherapy efficacy by increasing specificity? Collectively, our groundwork is paving the way for developing a human immune lexicon that is key to promoting effective bench-to-beside translation of findings.

Leveraging single-cell '*omics*' to unravel new insights into the human immune system

Achieving detailed understanding of the composition and function of the immune system at the fundamental unit of life — the cell — is essential to determining the prerequisites of health and disease. Historically, leukocyte populations have been defined by a combination of morphology, localization, functions, developmental origins, and the expression of a restricted set of markers. These strategies are inherently biased and recognized today as inadequate. Single-cell RNA sequencing (scRNAseq) analysis provides an unbiased, data-driven way of systematically detecting cellular states that can reveal diverse simultaneous facets of cellular identity, from discrete cell types to continuous dynamic transitions, which cannot be defined by a handful of pre-defined markers or for which markers are not yet known. We combine scRNAseq strategies together with in-depth follow-up profiling, phenotypic and functional characterization of prospectively isolated immune subsets defined by scRNAseq data to overcome such limitations. Our analyses of the human blood mononuclear phagocyte system resulted in the identification of six dendritic cell (DC), four monocyte, and one DC progenitor populations, thus revising the taxonomy of these cells (*Villani et al., Science 2017*). Noteworthy, five of these subsets had never been reported, illustrating the power of our integrative strategies to reopen the definition of these cell types. Our study highlighted the value of embarking on a comprehensive Human Cell Atlas initiative and offered a useful framework for conducting this kind of analysis on other cell types and tissues. We are currently contributing to the immune cell atlas effort by charting at high-resolution the human blood cellular landscape, and are studying paired human tissues with blood to better



Establishing a human blood dendritic cell and monocyte atlas. *We isolated ~2400 cells enriched from the healthy human blood lineage– HLA-DR+ compartment and subjected them to single-cell RNA sequencing. This strategy, together with follow-up profiling and functional and phenotypic characterization, led us to update the original cell classification to include six DCs, four monocyte subtypes, and one conventional DC progenitor.*

establish how circulating immune cells mirror those in tissue microenvironment in the context of health and disease.

We also continuously support development of in-depth expertise in single-cell 'omics' approaches, including single-cells strategies to map X-chromosome inactivation (Tukiainen, Villani, *Nature* 2017), new enrichment method targeting individual cell transcriptome in pooled library (Ranu, Villani, *Nucleic Acid Res* 2019), method's development to study single-T cells (Villani, *Methods Mol Biol* 2016) and application to study T cells infiltrates in tumor lesions (Izar *Science* 2016; Sade-Feldman,*Cell* 2019; Di Pilato, *Nature* 2019) and myeloid cell infiltrates (Olah M, *Nat Commun* 2018; Balan S, *Cell Rep* 2018; Chapuy L, *Mucosal Immunol* 2019).

Deciphering immune-related adverse events (irAEs) induced by immune-checkpoint inhibitor (ICI) therapy.

While ICI therapy is revolutionizing the treatment of solid cancers, its success is currently being limited by treatment-induced irAEs resembling autoimmune diseases that are affecting nearly every organ system. With ICI becoming first- and second-line of cancer treatments, it is expected that the number of irAEs will continue rising and limit immunotherapy efficacy unless we find solutions. Our multi-disciplinary translational group of scientists and clinicians are working towards developing a better understanding of the biological players and underlying molecular and cellular mechanisms involved in driving irAEs by directly studying patient blood and matched affected tissue samples using a range of systems immunology, immunogenomics and single-cell 'omics' strategies. Our translational research program may result in identifying putative cellular components and mechanisms that could be (i)targeted in a 'primary-prevention' approach to prevent irAE development, or (ii)targeted after onset of irAEs, without reducing the efficacy of the immunotherapy.

Selected Publications:

Villani AC, Sarkizova S, Hacohen N. Systems Immunology: learning the rules of the immune system. *Annu Rev Immunol* 2018; 36: 813-842.

Villani AC*†, Satija R*, Reynolds G, Sarkizova S, Shekhar K, Fletcher J, Griesbeck M, Butler A, Zheng S, Lazo S, Jardine L, Dixon D, Stephenson E, Nilsson E, Grundberg I, McDonald D, Filby A, Li W, De Jager PL, Rozenblatt-Rosen O, Lane AA, Haniffa M, Regev A†, Hacohen N†. Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes and progenitors. *Science* 2017; 356: 6335. pii: eaah4573.

Tukiainen T, **Villani AC**, Yen A, Rivas MA, Marshall JL, Satija R, Aguirre M,Gauthier L, Fleharty M, Kirby A, Cummings BB, Castel SE, Karczewski KJ, Aguet F, Byrnes A, GTEx Consortium, Lappalainen T, Regev A, Ardlie KG, Hacohen N, MacArthur DG. Landscape of X chromosome inactivation across human tissues. *Nature* 2017; 550(7675): 244-248.

Ranu N, **Villani AC**, Hacohen N, Blainey PC. Targeting individual cells by barcode in pooled sequence library. *Nucleic Acids Res 2019; 47(1): e4.*

Villani AC†, Karthik Shekhar†. Single cell RNA sequencing of human T cells. *Methods in Molecular Biology* 2017; 1514: 203-239.

Olah M*, Patrick E*, **Villani AC***, Xu J, White CC, Ryan KJ, Piehowski P, Kapasi A, Nejad P, Cimpean M, Connor S, Yung CJ, Frangieh M, McHenry A, Elyaman W, Petyuk V, Schneider JA, Bennett DA, De Jager PL, Brashaw EM. A transcriptomic atlas of aged human microglia informs neurodegenerative disease studies. *Nat Communications* 2018; 9(1): 539.

Di Pilato M, Kim EY, Cadilha BL, Prüßmann JN, Nasrallah MN, Seruggia D, Usmani SM, Misale S, Zappulli V, Carrizosa E, Mani V, Ligorio M, Warner RD, Medoff BD, Marangoni F, **Villani AC**, Mempel TR. Targeting the CBM complex causes T(reg) cells to prime tumours for immune checkpoint therapy. *Nature* 2019; 570(7759):112-116.

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