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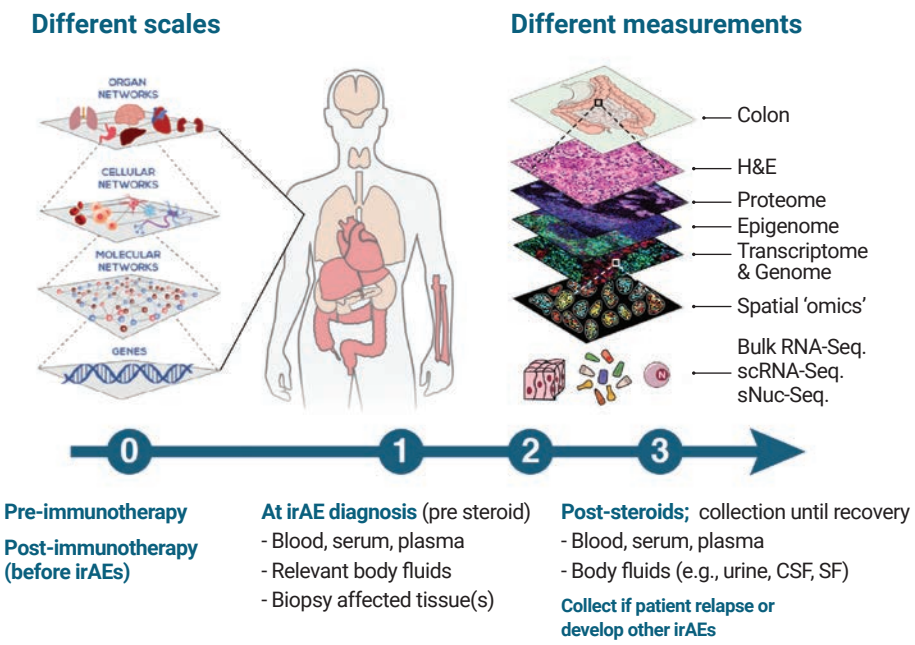
The Villani laboratory seeks to establish a comprehensive roadmap of the human immune system by achieving a higher resolution definition and functional characterization of cell subsets and rules governing immune response regulation, as a foundation to decipher how immunity is dysregulated in diseases. 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) and 'multi-omics' 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 establish how circulating immune cells mirror those in tissue microenvironment in the context of health and disease.

We also continuously support development



Overview of our strategy for exploring scale, time and modalities to discover underpinnings of diseases.

of in-depth expertise in single-cell 'multi-omics' experimental and computational strategies (Ding , *Nat Biotechnol* 2020; Li, *Nat Methods* 2020; Tukiainen, Villani, *Nature* 2017; Ranu, Villani, *Nucleic Acid Res* 2019; Villani, *Methods Mol Biol* 2016), and its application to study immune cells infiltrates in healthy, tumor lesions and inflamed tissue (Izar, *Science* 2016; Sade-Feldman, *Cell* 2019; Di Pilato, *Nature* 2019; Olah, *Nat Commun* 2018; Balan, *Cell Rep* 2018; Popescu, *Nature* 2019; Smillie, *Cell* 2019; Abbas, *Nat Immunol* 2020; Delorey, *Nature* 2021; Alladina, *Science Immunol* 2023).

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 irAE incidence 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 (Zubiri, *J Immunother Cancer* 2021; Thomas, *Nature Med* 2023). 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, and/or (ii) targeted after onset of irAEs, without reducing the efficacy of the immunotherapy.

Selected Publications:

Thomas MF*, Slowikowski K*, Manakongtreecheep K, Pritha Sen, ..., Li B, Reynolds KL†, **Villani AC**†. Altered interactions between circulating and tissue-resident CD8 T cells with the colonic mucosa define colitis associated with immune checkpoint inhibitors. *Nature Medicine* 2023 (In Press).

Alladina J*, Smith NP*, Kooistra T, Slowikowski K, Kernin IJ, ..., Luster AD, **Villani AC**†, Cho JL†, Medoff BD†. A human model of asthma exacerbation reveals transcriptional programs and cell circuits specific to allergic asthma. *Sci Immunol*. 2023; 8(83): eabq6352.

Villani AC. The evolving landscape of immune-related adverse events that follow immune checkpoint immunotherapy in cancer patients. *Immunol Rev*. 2023 Aug 26.

Zubiri L, Molina GE, Mooradian MJ, ... , Semenov YR, **Villani AC**†, Reynolds KL†. Effect of a multidisciplinary Severe Immunotherapy Complications Service on outcomes for patients receiving immune checkpoint inhibitor therapy for cancer. *J Immunother Cancer*. 2021; 9(9): e002886.

Delorey TM*, Ziegler CGK*, Heimberg G*, Normand R*,..., Shalek AK†, **Villani AC**†, Rozenblatt-Rosen O†, Regev A†. COVID-19 tissue atlases reveal SARS- CoV-2 pathology and cellular targets. *Nature* 2021; 595(7865):107-113. PMID: 33915569.

Villani AC*, Satija R*, Reynolds G, ... , 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.

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