COMPREHENSIVE SINGLE-CELL IMMUNE PROFILING OF LYMPHOID AND PERIPHERAL TISSUES OF AGED MICE USING HIGH-PARAMETER FLOW CYTOMETRY BY CYTOF TECHNOLOGY

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Background
High-parameter flow cytometry is a major tool used in human and mouse studies to discover novel immunological mechanisms of infectious diseases, cancer and immunosenescence. Fluorescence-based cytometry can be especially challenging in mouse model studies due to the large amount of cell samples needed for single-stained controls. CyTOF® technology has transformed high-parameter flow cytometry by enabling 50-plus-marker analysis per tube of sample, with easy panel design and no need for single-stained or autofluorescence controls. Flow cytometry by CyTOF thus provides an efficient and unbiased approach to discovering novel cell populations and unique functional states of immune cells with minimal cell number requirements. Here we present how Standard BioTools™ commercially available mouse antibody products can be incorporated into a high-parameter panel, enabling comprehensive single-cell immune profiling of both lymphoid and peripheral tissues of individual aged mice.

Methods
Antibodies from the Maxpar® and Maxpar OnDemand™ catalogs were used to create a panel identifying lymphocytes, myeloid cells, functional markers and immune checkpoint markers (for example, PD-1 and PD-L1). Lymphoid and peripheral tissues (for example, lung and spleen) from young adult and aged mice were harvested and processed for cell staining with a 42-parameter cytometry panel. Sample acquisition was performed using the Helios™ mass cytometer. High-dimensional single-cell data analysis was carried out with Maxpar Pathsetter™ and R package PhenoGraph and UMAP.

Results
In young adult and aged mice, we identified more than 10 major immune cell populations, including lymphocytes and myeloid cells in multiple lymphoid and peripheral tissues, by unsupervised high-dimensional data clustering. We further defined and quantified more than 30 immune cell clusters with distinct phenotypes (for example, naive, effector, effector memory and central memory T cells). The frequency, activation and checkpoint marker status of these cell clusters were analyzed and compared between young adult and aged mice to identify the age-related changes in the immune system of multiple organs.

Conclusions
This work demonstrates that by using CyTOF technology together with Maxpar and Maxpar OnDemand mouse antibodies, comprehensive single-cell phenotyping of the immune system in multiple distinct tissues of individual aged mice is easily accomplished. Moreover, the high-parameter panel can be further customized for deep functional characterization of specific lymphocytes and myeloid cell subsets. Thus, by utilizing the well-curated collection of Maxpar and Maxpar OnDemand mouse antibodies, flow cytometry by CyTOF can significantly facilitate the mechanistical studies of mouse models and expand our understanding of age-related human diseases, infection and cancer.