expression of cytolytic proteins and modulate the immune response through costimulatory molecules.

Materials and Methods In multiple myeloma, malignant plasma cells accumulate in the bone marrow through clonal expansion, crowding out other cells and leading to anemia, renal insufficiency, immunosuppression, and increasing risk of multisystem organ damage if untreated. Cellular and antibody-mediated immunotherapeutic approaches, including CAR T cells and monoclonal antibodies targeting CD38, have been developed to treat multiple myeloma. Since NK cells can also indirectly impact CAR T cell or antibody-based immuno-therapies, characterizing these cells using optimized and reproducible assays is critical.

Results CyTOF®is a high-plex flow cytometry technology that uses metal-isotope-taggedantibodies to probe cellular phenotypes and functions. In contrast tofluorescence-based conventional and spectral flow cytometry, CyTOF experimental workflows are streamlined because autofluorescence is not an issue and signal spillover is minimal, allowing rapid design and application of 40-plus-marker panels. To expand on the increasing clinical and preclinical utility of the 30-marker Maxpar® Direct™ Immune Profiling Assay™ (Maxpar Direct Assay), we developed 9 add-on Expansion Panels for deeper phenotyping of specific cell types and activation states, including panels designed to characterize ex vivo and activated myeloid cells, T cells, and NK cells.

Conclusions Here we demonstrate combining the Maxpar Direct Immune Profiling Assay with the NK Cell Expansion Panel (CD181, NKp30, NKp46, PD-1, NKG2A, ICOS, and TIGIT) or the T Cell Expansion Panel 3 (OX40, TIGIT, CD69, PD-1, Tim-3, ICOS, and 4-1BB) with the Basic Activation Expansion Panel (IL-2,TNFα, IFNγ, CD107a, perforin, granzyme B) to enable deep immunoprofiling of multiple myeloma PBMC.

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P02.11 50-PARAMETER FLOW CYTOMETRY BY CYTOF EMPOWERS COMPREHENSIVE SINGLE-CELL IMMUNE PROFILING OF PULMONARY IMMUNOSENESCENCE IN AGED MICE

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Background Immuneprofiling by flow cytometry is essential to uncovering novel immunological mechanisms of cancer, infections, autoimmunity, and immunosenescence. Fluorescence-based flow cytometry can be challenging due to spectral overlap, autofluorescence, parameter limitation, and the large number of cells needed for single-stained controls. CyTOF® flow cytometry (CyTOF) utilizesmetal-tagged antibodies and goes beyond the limitations of fluorescence to simultaneously detect 50-plus markers per tube of sample with easy panel design and no need for single-stained or autofluorescence controls. CyTOF provides an efficient and unbiased approach to discovering novel subsets and unique functional states of immune cells, maximizing insights from precious samples.

Materials and Methods The Maxpar® OnDemand Mouse Immune Profiling Panel Kit (Mouse Immune Profiling Panel) was designed for CyTOF to provide an efficient and comprehensive end-to-end solution for mouse immune profiling studies. The Mouse Immune Profiling Panel comprises 33 antibodies targeting key lineage and functional surface markers to characterize at least 38 lymphocyte and myeloid cell populations in mouse tissues. In support of numerous metal-tagged antibodies provided by Standard BioTools™, the Mouse Immune Profiling Panel can be customized into various high-parameter panels to meet various needs of mouse studies. Based on the Mouse Immune Profiling Panel, preset templates for automated analysis were designed by Standard BioTools to provide easy and unbiased immune profiling analysis by Maxpar Pathsetter™ software.

Results Immunosenescenceperturbs lung cancer onset and development, yet the mechanisms remain largely unknown. To identify aging-associated immune alteration in mice, the Mouse Immune Profiling Paneland 10 Maxpar antibodies were utilized to develop a 50-parameter CyTOF panel (Figure 1A) to characterize and compare 55 pulmonary immune subsets between aged and young adult mice (Figure 1B). Maxpar Pathsetter software was used for automated high-dimensional analysis.

Conclusions This study highlights how StandardBioTools mouse antibody products and analytical tools can be incorporated to enable comprehensive single-cell immune profiling of mouse models.

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P02.12 IMMUNO-ONCOLOGY IMAGING MASS CYTOMETRY STUDY OF THE STRUCTURAL AND CELLULAR COMPOSITION OF THE TUMOR MICROENVIRONMENT IN HUMAN CANCERS

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Background High-plex imaging is becoming an increasingly valuable tool to study the tumor microenvironment (TME) in immuno-oncology. The ability to evaluate the multiparametric response in the TME is crucial to predict therapeutic drug efficacy. Particularly, assessment of immunological and oncological processes that dictate tumor growth, metastasis, and immune response are essential for identifying candidates for further clinical evaluation.

Materials and Methods Imaging Mass Cytometry™ (IMC™) permits analysis of 40-plus distinct tissue and cellular markers simultaneously on tumor samples, providing a thorough evaluation of the spatial landscape of the TME. Application of IMC to study cancer has facilitated important discoveries regarding the interplay of tumor and immunecells in the TME. Here, we demonstrate how our reagents and technology support these applications.
Results Through systematic selection of human antibodies from both the Maxpar® catalog and Maxpar OnDemand™, we have customized panel kits that permit qualitative and quantitative evaluation of critical tumor pathophysiological parameters. Furthermore, we present a consolidated single-cell analysis (SCA) pipeline by combining the panel kit with the Maxpar IMC Cell Segmentation Kit (ICSK) and present quantitative evaluation of the cellular and structural landscape of theTME in various human cancers.
Conclusions Overall, customization of the Human Immuno-Oncology IMC Panel Kit successfully defines the tissue architecture of the TME, metastatic and growth potential of tumor cells, and immune cell phenotype and activation in human tumors.

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P02.13 IDENTIFYING PATHOPHYSIOLOGICAL FEATURES OF MOUSE TUMORS USING IMAGING MASS CYTOMETRY

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Background Mouse tumor models have greatly enhanced our understanding of tumorigenesis and are widely utilized as the preferred model organism for cancer studies and preclinical drug testing. The ability to evaluate the multiparametric response in the tumor micro-environment (TME) is crucial to predict therapeutic drug efficacy. Particularly, assessment of immunological and oncological processes that dictate tumor growth, metastasis, and immune response are essential for identifying candidates for further clinical evaluation.
Materials and Methods Imaging Mass Cytometry™ (IMC™) permits analysis of 40-plus distinct tissue and cellular markers simultaneously on tumor samples, providing a thorough evaluation of the spatial landscape of the TME. Application of IMC to study cancer has facilitated important discoveries regarding the interplay of tumor and immune cells in the TME. Here, we introduce the Standard BioTools™ **Maxpar® OnDemand Mouse Immuno-Oncology IMCPanel Kit** (PN 9100005) designed for high-parameter preclinical immuno-oncology studies.
Results Through systematic selection of mouse antibodies from both the Maxpar catalog and from Maxpar OnDemand™, we have created panel kits that permit qualitative and quantitative evaluation of critical tumor pathophysiological parameters. This poster describes the design and application of an IMC panel consisting of 4 modular subpanels and defines their performance on multiple mouse tumor models. Furthermore, we present a consolidated single-cell analysis (SCA) pipeline by combining the panel kit with the Maxpar IMC Cell Segmentation Kit (ICSK, PN201500) and present quantitative evaluation of the cellular and structural landscape of mouse non-small-cell lung cancer (NSCLC), B cell lymphoma, colon adenocarcinoma, and renal carcinoma TME.
Conclusions Overall, application of the **MouseImmuno-Oncology Panel** successfully defines the tissue architecture of the TME, metastatic and growth potential of tumor cells, and immune cell phenotype and activation in mouse tumors.

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P02.14 HIGH-PLEX CO-DETECTION OF RNA AND PROTEIN TO EXPLORE TUMOR-IMMUNE INTERACTIONS UTILIZING RNASCOPE WITH IMAGING MASS CYTOMETRY

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Background The next breakthroughs in immuno-oncology will be driven by high-plex tools that decipher the spatial arrangement of different cell types within the tumor microenvironment (TME).
Materials and Methods Imaging Mass Cytometry™ (IMC™) is a proven tool for the study of complex cellular interactions in the TME. It utilizes CyTOF®technology for simultaneous assessment of 40-plus protein markers at subcellular resolution without spectral overlap or background autofluorescence, thus providing unprecedented insight into the organization and function of the TME. Despite this, some protein targets are challenging to include in IMC as they have very few or no commercial antibodies available. Moreover, although cellular identity can easily be deciphered through detection of protein targets, knowledge of the cell's transcriptome improves understanding of cellular function and activation state.
Results Here,we present a robust and reliable workflow that combines the highly sensitive and specific RNAscope™ technology for RNA detection with the multiplexing capability of IMC to visualize key RNA and protein markers in the same tumor samples.
Conclusions The RNAscopeHiPlexv2 assay was combined with protein detection using IMC to evaluate expression of both RNA and protein targets in formalin-fixed, paraffin-embedded (FFPE) tumor tissue microarray (TMA).

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