

undergoes weaning-induced involution and supports breast cancer metastasis. *Cancer Discov* 2017;**7**(2):177–187.

5. Shi FD, Ljunggren HG, La Cava A, Van Kaer L. Organ-specific features of natural killer cells. *Nat Rev Immunol* 2011;**11**(10):658–671.

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#### MULTIOMIC CHARACTERIZATION OF T-CELL POPULATIONS AT THE SINGLE-CELL LEVEL UTILIZING SENSITIVE DEXTRAMERS AND BD<sup>®</sup> ABSEQ ON THE BD RHAPSODY™ SINGLE-CELL ANALYSIS SYSTEM

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**Background** Adoptively transferred antigen-specific T cells have shown great efficacy in treatment of some virus-associated diseases and malignancies. A major driver of the development of adoptive T-cell therapy has been our ability to successfully characterize the functional status and antigen specificity of T cells. However, this has been limited by inefficient detection of antigen-specific T cells possibly due to their low frequency and low binding affinities to known MHC-peptide complexes.

**Methods** Here, we aim to combine two powerful technologies, advanced dCODE™ Dextramer<sup>®</sup> from Immudex and single-cell multiomics analysis using the BD Rhapsody™ Single-Cell Analysis system, to detect and characterize disease-specific CD8+ T cells within thousands of PBMCs.

**Results** Currently, we are able to identify over 350 mRNAs alongside a panel of over 20 BD<sup>®</sup> AbSeq cell surface protein markers which can be associated with T cell activation states. These data can be used to define T-cell phenotypes alongside antigen specificity of enriched CD8+ Dextramer(R)+ cells from a PBMC population.

**Conclusions** his study outlines our ability for high-resolution T-cell profiling that has broader implications and utility in immuno-oncology, infectious diseases and autoimmunity.

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#### TUMOR-SPECIFIC CYTOLYTIC CD4 T CELLS MEDIATE PROTECTIVE IMMUNITY AGAINST HUMAN CANCER

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**Background** CD4 T cells have been implicated in cancer immunity for their helper functions. However, their direct cytotoxic potential remains elusive in cancer patients. Here, we aimed at assessing the presence, rate and cytotoxic function of tumor-specific Th-CTX directly in cancer patients.

**Methods** We capitalized on published single cell transcriptomic analyses of patient samples, integrated with the direct phenotypic and functional characterization of clonal, tumor-specific CD4 T cell populations, using peptide-MHC class II multimers

and a novel high-throughput single-cell cytotoxicity assay in picowell arrays. The direct tumor cell killing by cytolytic tumor-specific CD4 T cells in the arrays was monitored in a high-throughput manner by combining multi-channel time-lapse microscopy with deep neural networks.

**Results** By mining single-cell RNA-seq datasets of tumor infiltrating lymphocytes, we identified CD4 T cells displaying cytotoxic phenotypes in different human tumors. The cytolytic CD4 T cells formed a distinct cluster and expressed genes related to classical cytotoxic functions, largely resembling CD8 T cell gene profiles. Using the peptide MHC class II multimer technology, we confirmed directly ex vivo the presence of cytolytic tumor antigen-specific CD4 T cells, both in the circulation and in the tumors of patients. We performed an integrated phenotypic and functional characterization of cytolytic tumor-specific CD4 T cells, down to the single cell level, through a high-throughput nanobiochip consisting of massive arrays of picowells with sub-nanoliter volumes and machine learning. We demonstrated a direct, contact-dependent, granzyme-dependent cytotoxic activity against tumor cells, with delayed kinetics compared to classical cytotoxic lymphocytes. Lastly, we discovered that this cytotoxic activity was at least in part dependent on the expression of SLAMF7, a homophilic receptor known to regulate NK cell activity.

**Conclusions** Our work provides a deep characterization of human Th-CTX in cancer and supports their role in tumor immunity. Moreover, our results showing that agonistic engagement of SLAMF7 enhances the cytolytic capacity of tumor-specific CD4 T cells, suggests that targeting these cells might prove synergistic with the use of other immunotherapies in cancer patients.

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#### THE DIFFERENTIATION STATUS OF SYSTEMIC PD1+ CD8 T CELLS IS ASSOCIATED WITH FAVORABLE OUTCOME TO PD1 BLOCKADE THERAPY IN NON SMALL CELL LUNG CANCER

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**Background** Non small cell lung cancer is one of the cancer types where Immune checkpoint blockade has demonstrated unprecedented clinical efficiency. However, only a fraction of patients benefit from such therapy; factors determining this response are yet to be elucidated. Here, we investigated whether the differentiation status of circulating CD8 T cells might be associated with outcome of PD1 blockade therapy in NSCLC.

**Methods** We used multi-parameter flow cytometry to study CD8 T cell differentiation states in NSCLC patients at baseline and to examine the effects of blocking the PD1/PDL1 pathway on those cells.

**Results** We found that responders to PD1 blockade therapy has more peripheral PD1+ CD8 T cells with an early-like differentiated status at baseline and that this phenotype is associated with longer survival. Moreover, PD1 blockade induced reinvigoration is mostly observed in cells with this with an early-like differentiated status.

**Conclusions** An early like differentiation status of peripheral CD8 T cells is associated with favorable outcome of PD1 blockade immunotherapy

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