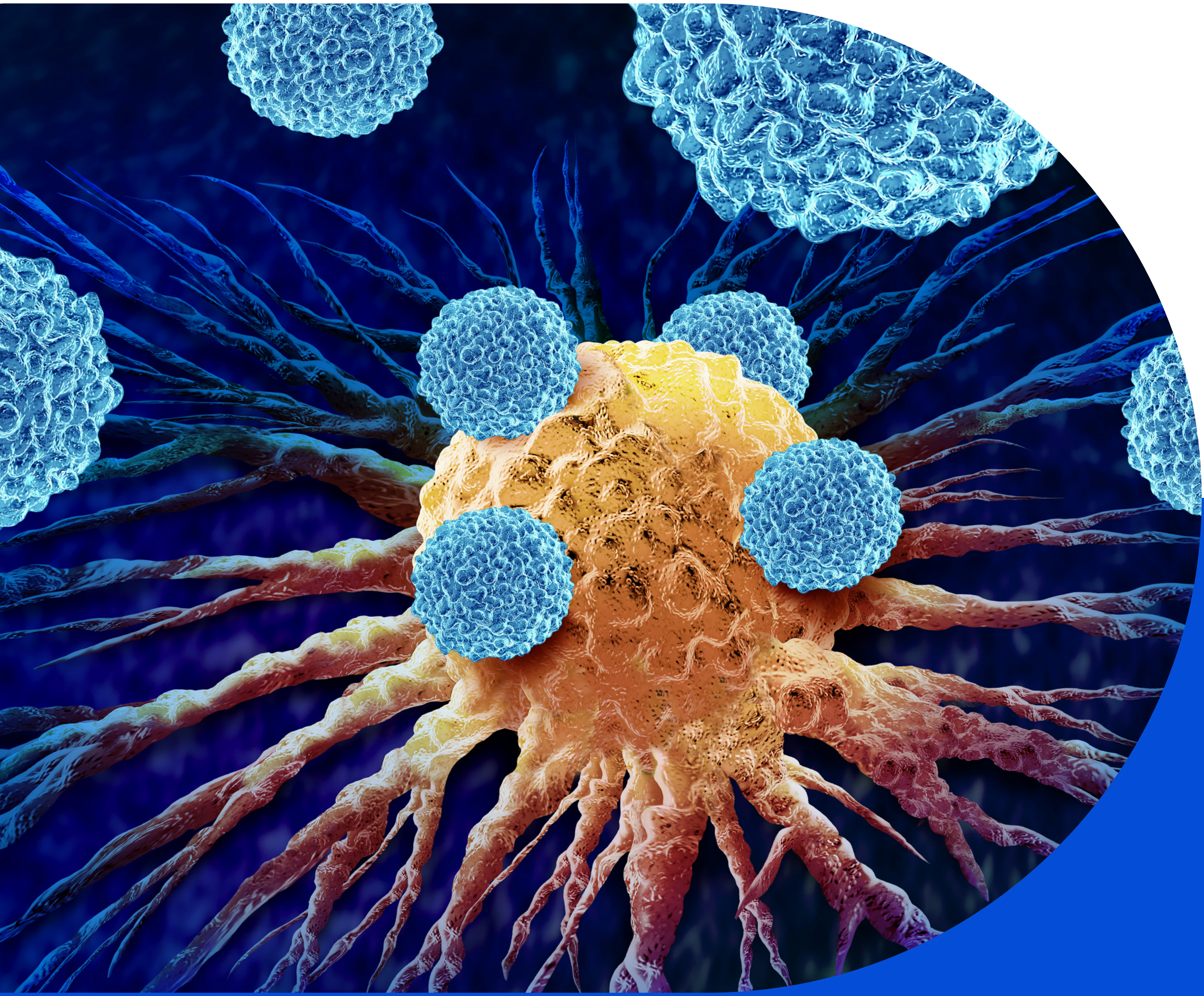

Advancing immuno-oncology through single-cell multiomics

BD

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Introduction

Single-cell multiomics has emerged as an indispensable tool in advancing our understanding of immuno-oncology. This approach transcends traditional bulk analyses by enabling the simultaneous examination of various omic layers within individual cells. Traditionally, single-cell RNA (scRNA)-seq paved the way, offering glimpses into the transcriptomic realm. However, recent innovations utilizing oligo-barcoded antibodies have ushered in a new era of multiomic analysis, enabling the simultaneous exploration of proteins alongside mRNA. In the context of immune response in cancer, it unravels a nuanced tapestry of cellular heterogeneity, unveiling distinct phenotypic profiles and functional states of immune cells within the tumor microenvironment (TME). By integrating genomics, transcriptomics and proteomics at the single-cell level, researchers are gaining unprecedented insights into the dynamic interplay between tumor cells and immune effectors, including those associated with cancer and cancer immunotherapies.

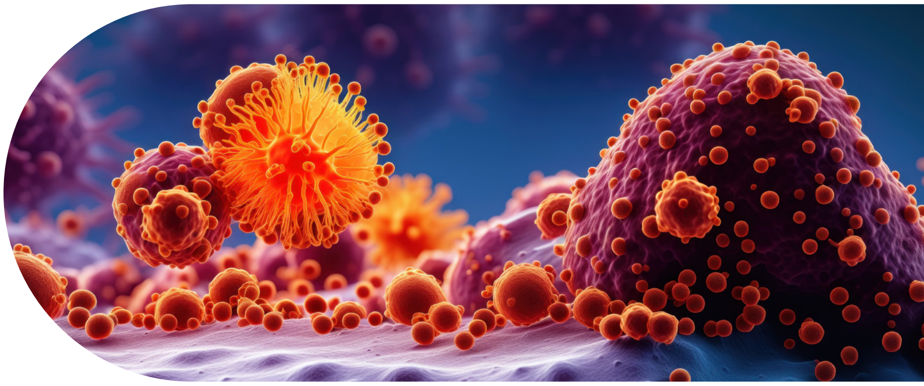
Cancer immunotherapies have the potential to induce durable anti-tumor immune responses; however, the efficacy of these treatments varies among patients and partially depends on the complex interplay between malignant and non-malignant cells within the TME, which includes T cells, B cells, tumor-infiltrating lymphocytes (TILs) and a range of other cell types. A comprehensive view of the cellular terrain involved in cancer progression and suppression reveals the molecular mechanisms underpinning immune evasion, activation and response in normal and disease states. Additionally, single-cell multiomics has the potential to unveil biomarkers that can propel immunotherapy research.

This book will take you on a comprehensive journey through the field of immuno-oncology, focusing on the instrumental role the BD Rhapsody™ Single-Cell Analysis System plays and how it is used to conduct single-cell multiomics experiments that push the field forward. Each of the five studies summarized here uses BD reagents and equipment to conduct single-cell multiomic experiments whose results provide insight into the complexity of the phenomenon of cancer. The five studies all use a similar experimental process:

1. Collect/enrich samples
2. Stain samples with some or all of the following: BD® Single-Cell Multiplexing Kit/BD® AbSeq Antibody-Oligos/fluorochrome antibodies
3. Conduct cell sorting with the BD FACSymphony™ S6 Cell Sorter or BD FACSMelody™ Cell Sorter
4. Run samples through the BD Rhapsody™ Single-Cell Analysis System
5. Prepare samples for next-generation sequencing
6. Sequence samples with next-generation sequencing
7. Analyze data with any of the following software: the BD Rhapsody™ Sequence Analysis Pipeline/SeqGeq™ Software/R package

Introduction

Through the lens of five informative chapters, we delve into a diverse array of studies, each illuminating a distinct facet of this dynamic field.



01 **In chapter 1**, we look at chronic lymphocytic leukemia (CLL), the most prevalent form of leukemia in adults. We navigate the intricate landscape of CLL pathogenesis, where bulk RNA-seq studies, though insightful, fall short in deciphering intratumoral heterogeneity. **Harnessing the power of single-cell RNA sequencing via the BD Rhapsody™ System, this chapter sheds light on disease-specific signatures, offering a more granular view.**

02 **Chapter 2** deals with B cell malignancies, a dynamic arena characterized by the interplay between malignant cells and the TME. The focus centers on the B and T lymphocyte attenuator (BTLA) within a murine model of B cell lymphoma. **Through a high-throughput sorting strategy and single-cell multiomic analysis using the BD Rhapsody™ System, this chapter reveals the interplay between neoplastic cells and their surroundings.**

03 **Chapter 3** delves into hematopoiesis, a choreographed process that underlies the generation of diverse blood cell lineages. In the shadow of cancer, the orchestration falters, giving rise to immunosuppressive myeloid cells. This chapter unravels the mechanisms driving tumor-induced perturbations in hematopoiesis. **It examines sorted hematopoietic stem and progenitor cell (HSPC) populations through the lens of a melanoma mouse model, providing a comprehensive understanding of the alterations induced by the tumor.**

04 **Chapter 4** unveils critical roles of T and B cells in anti-tumor activity, spotlighting their unique antigen receptors and clonotypes. By studying the T cell receptor (TCR) and B cell receptor (BCR) repertoire in the tumor microenvironment, this chapter exposes the intricate dynamics governing immune responses to cancer. **Using an mc38 syngeneic carcinoma tumor model, this chapter showcases the power of the BD Rhapsody™ System in unraveling the heterogeneity of T and B cells and their clonotype diversity.**

05 **Chapter 5** highlights advances in single-cell multiomics that allow high-resolution functional characterization of intratumoral immune cells. In-depth dissection of the immune composition of the tumor microenvironment using both flow cytometry and the BD Rhapsody™ System allows examination of the expression of mRNA and surface protein markers at single-cell resolution. **Understanding the transcriptional and proteomic phenotypes of tumor infiltrating leukocytes is helpful for development of novel immunomodulatory strategies for cancer.**

Single-cell multiomics experiments such as these are key to unlocking the secrets of cancer and paving the way for better treatments. Together, these chapters form a knowledgebase to help unravel the complexities of immuno-oncology. This book serves to guide researchers in their pursuit of understanding and combating cancer at the single-cell level.

CHAPTER 1

Exploring tumor heterogeneity of chronic lymphocytic leukemia in humans

This study demonstrates the power of single-cell analysis in unraveling the complexities and heterogeneity of CLL, providing valuable insights for understanding its pathogenesis and potentially informing treatment strategies.

Chronic lymphocytic leukemia (CLL) is the most common leukemia in adults and is characterized by the proliferation and accumulation of neoplastic B cells in the peripheral blood, bone marrow and secondary lymphoid organs. Numerous studies have revealed the heterogeneity and complexity underlying the pathogenesis of CLL. While bulk RNA-seq studies provide frameworks to understand CLL pathogenesis, transcriptome data from a complex mixture of cells derived from bulk tumor samples fail to accurately elucidate the intratumoral heterogeneity of CLL. The emergence of single-cell RNA sequencing technology provides an unprecedented opportunity to discover disease-specific signatures and reveal the heterogeneity of CLL.

This study serves as an example of simultaneous measurement of protein and mRNA expression at single-cell resolution using the BD Rhapsody™ Single-Cell Analysis System on healthy and clinical research CLL samples. The researchers stained blood cells simultaneously with the BD® Human Single-Cell Multiplexing Kit (SMK), BD® AbSeq Antibody-Oligos and fluorochrome-labeled antibodies and then used the BD FACSMelody™ Cell Sorter to isolate CD19 cells. They then used a single-cell multiomics workflow on the BD Rhapsody™ System to conduct an in-depth examination of mRNA and surface protein expression, which revealed disease-specific signatures.

Find other researchers studying leukemia using the BD Rhapsody™ System technology:

- Triana S, et al. *Nat Immunol*. 2021;22(12):1577-1589.

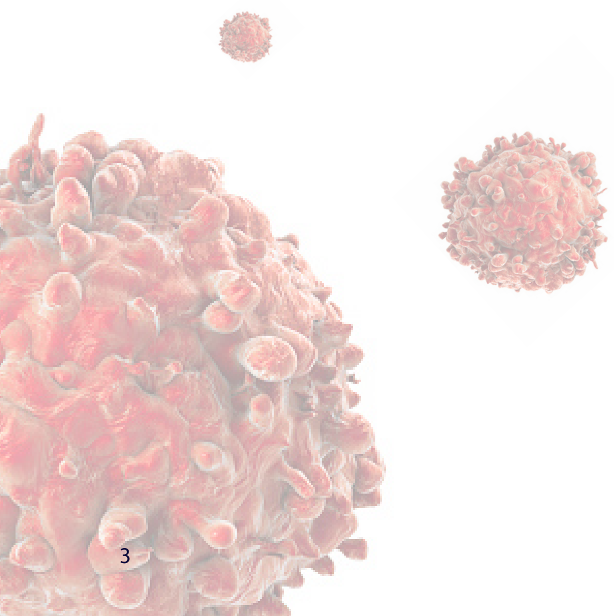
  Single-cell proteo-genomic reference maps of the hematopoietic system enable the purification and massive profiling of precisely defined cell states

- Ediriwickrema A, et al. *Blood*. 2021;138(Supplement 1):3259-3259.

  Single cell analysis of adult human hematopoietic stem and progenitor cells identifies a novel lymphoid primed multipotent progenitor that expands in relapsed acute myeloid leukemia

- Xu X, et al. *Hematology*. 2023;28(1):2240129.

  Analysis of single-cell sequencing results of an elderly patient with myeloid leukemia reveals high expression of multiple oncogenes in monocytes and hematopoietic stem cells



Results

t-SNE analysis was performed on four CLL samples and three healthy donor samples using mRNA expression, surface protein expression or a combination of both. t-SNE plots generated based on BD® AbSeq Panel data showed higher level of separation among different samples compared to using RNA-seq data alone. A combination of both mRNA and BD® AbSeq Panel data greatly improved the resolution of different samples (Figure 1A). The healthy samples overlapped to a greater extent, showing less variability (Figure 1A, right), whereas the four CLL samples formed distinct clusters individually indicating high cellular heterogeneity, suggesting the clinical course of CLL patients can be heterogeneous. CLL is characterized by clonal expansion and accumulation of $CD5^+$ B lymphocytes. Additionally, malignant B cells in CLL patients can also express CD11c at higher levels. BD® AbSeq Antibodies revealed higher expression of CD11c and CD5 in CLL samples compared to healthy donors, correlating to pathogenesis of CLL (Figure 1B). On the other hand, low CD10 and CD20 expression were observed in CLL samples compared to healthy donors, which is consistent with published literature (Figure 1B).

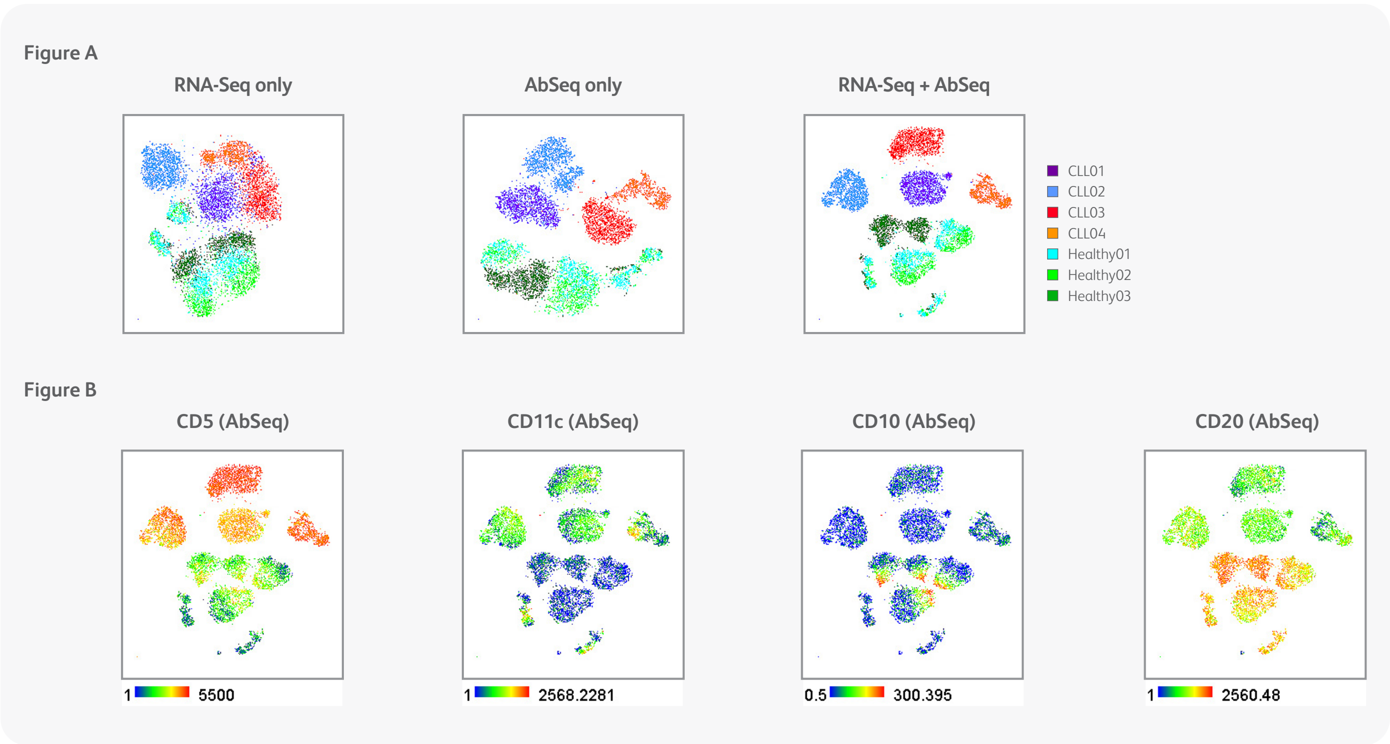


Figure 1. Multiomic analysis of CLL samples and healthy donors. A) t-SNE clustering of different samples using mRNA or surface protein or a combination of both. **B)** t-SNE coordinates of key markers associated with CLL disease progression based on BD® AbSeq Antibodies.

To understand the gene signatures underlying the distinct clustering between healthy and CLL samples, the researchers performed a differential expression analysis using single-cell mRNA and BD® AbSeq Antibody data. The analysis revealed three groups of differentially expressed genes in healthy samples and four groups in the CLL samples (Figure 2). Among these groups, aberrant expression of markers like CD5 and CD11c in CLL indicated potential clinical complexities and secondary tumors.

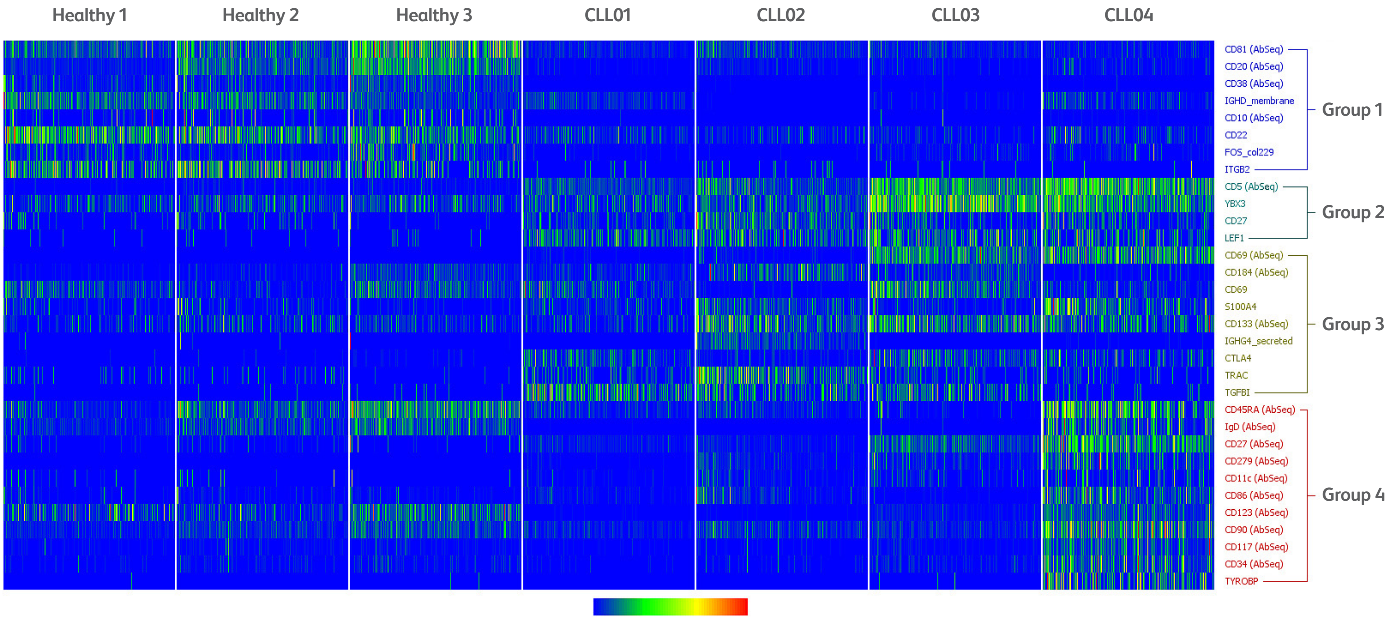
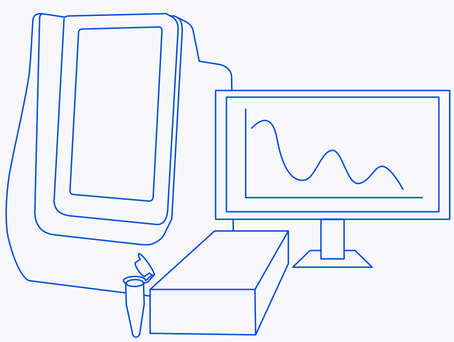


Figure 2. Heatmap of representative differentially expressed genes or surface proteins in CLL and healthy samples at the single-cell level. Expression of genes or proteins (indicated as AbSeq) was lower (Group 1) or higher (Group 2 and 3) in CLL samples compared to healthy samples. A subset of genes or proteins whose expression was specifically associated with CLL04 is also listed (Group 4).

BD products that enabled this study:



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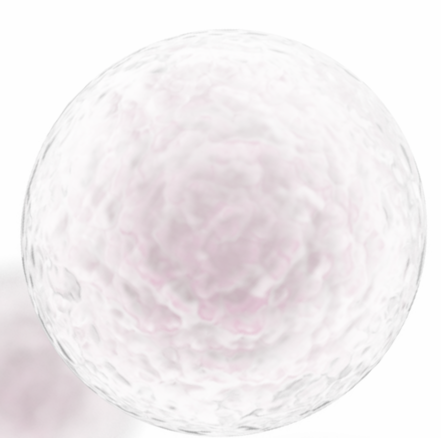
CHAPTER 2

In-depth characterization of CD8⁺ T cells to determine changes in protein and mRNA expression in a murine model of B cell lymphoma induction

This research provides insights into the complex interplay of immune cell populations in response to B cell lymphoma. The results presented here reveal methods for identifying therapeutic targets in B cell lymphoma.

The pathogenesis of B cell malignancies is a dynamic process involving reciprocal interactions between malignant cells and surrounding components in the tumor microenvironment. In the case of certain B cell lymphomas, neoplastic B cells migrate to lymphoid sites and prompt immune cell infiltration. The infiltration of cytotoxic lymphocytes correlates with tumor cell killing and good prognosis. However, most immune cell types in the tumor niche often become unresponsive or even end up promoting tumor growth.

This study showcases a high-throughput sorting strategy that isolated six lymphocyte populations and subsequently analyzed them using single-cell multiomics. The experiments are designed to examine the role of BTLA in tumor progression, revealing changes in protein and mRNA expression, particularly in CD8⁺ T cells.



Find other researchers studying lymphoma using the BD Rhapsody™ System technology:

- Sarén T, et al. *Clin Cancer Res*. 2023;29(20):4139-4152.

  Single-cell RNA analysis reveals cell-intrinsic functions of CAR-T cells correlating with response in a phase II study of lymphoma patients

- Kruglov O, et al. *Cancer Immunol Immunother*. 2022;71(4):919-932.

  The pivotal role of cytotoxic NK cells in mediating the therapeutic effect of anti-CD47 therapy in mycosis fungoides

- Kita K, et al. *Australasian Journal of Dermatology*. 2022;63:e150-e154.

  Single-cell RNA sequencing of mycosis fungoides reveals a cluster of actively proliferating lymphocyte

Results

Researchers induced tumors by inoculating C57BL/6 mice with an ovalbumin-expressing B cell lymphoma cell line. Then, to investigate tumor-specific immune responses, Btla+/+ or Btla–/– ovalbumin-specific CD8$^+$ T cells (OT-1 T cells) were adoptively transferred to the mice seven days later. Single-cell suspensions of splenocytes were stained with the BD® Mouse Immune Single-Cell Multiplexing Kit (SMK) for sample multiplexing, 34 BD® AbSeq Ab-Oligos and 21 fluorescent antibodies.

Six cell populations were simultaneously isolated using the BD FACSymphony™ S6 Cell Sorter, including the transferred OT-1 T cells, endogenous CD8$^+$ T cells, effector CD4$^+$ T cells, NK cells and two myeloid cell subsets containing Ly6g high Ly-6C low or Ly-6G low Ly-6C high myeloid-derived suppressor cells. After sorting of cells from the Btla+/+ and Btla–/– OT-1 groups, the 12 sorted populations were pooled together prior to being loaded onto the BD Rhapsody™ System to conduct single-cell multiomics analysis. The strategy allowed separation of endogenous CD8$^+$ T cells from OT-1 T cells during cell sorting and downstream analysis.

The researchers performed an in-depth characterization of CD8$^+$ T cells to determine changes in protein and mRNA expression upon lymphoma induction. Differential gene and protein expression analysis between transferred OT-1 T cells from the Btla+/+ and Btla–/– groups revealed a higher CD279 (PD-1) protein expression in the knockout cells (Figure 1A, heatmap). CD279 signaling controls T cell-mediated immune responses, playing critical roles in tumorigenesis. Thus, this result prompted the scientists to investigate the activation status of endogenous CD8$^+$ T cells as well.

Figure A

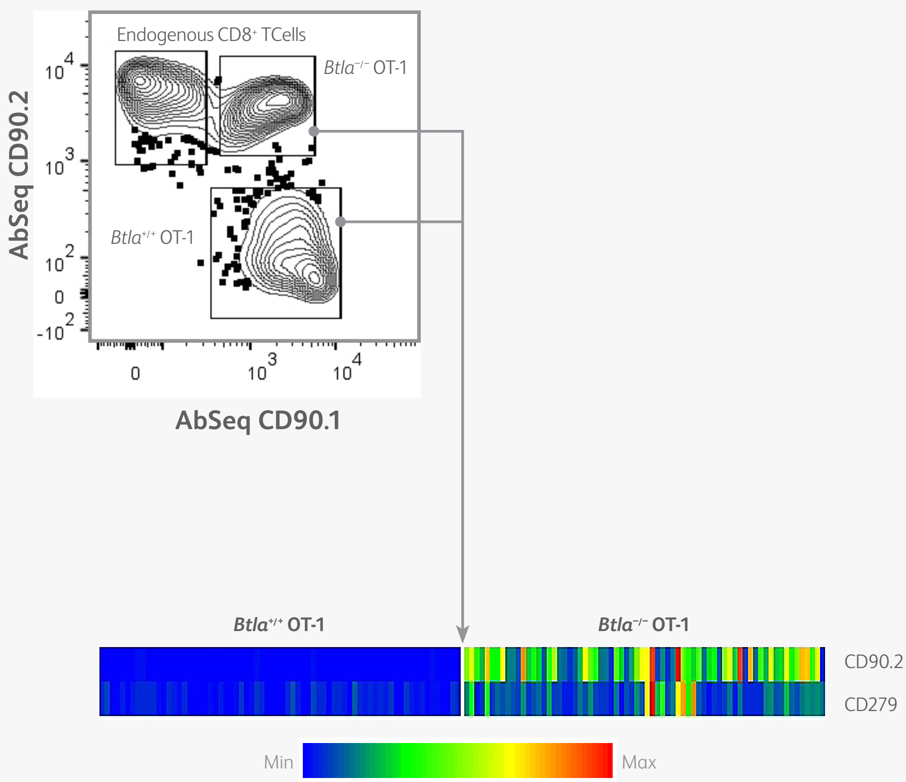


Figure 1. Analysis of the differences between transferred OT-1 T cells and endogenous CD8+ T cells from lymphoma-bearing mice. A) Contour plots and heatmap showing differential protein expression of Btla+/+ and Btla–/– OT-1 T cells segregated based on CD90.1 and CD90.2 surface protein expression.

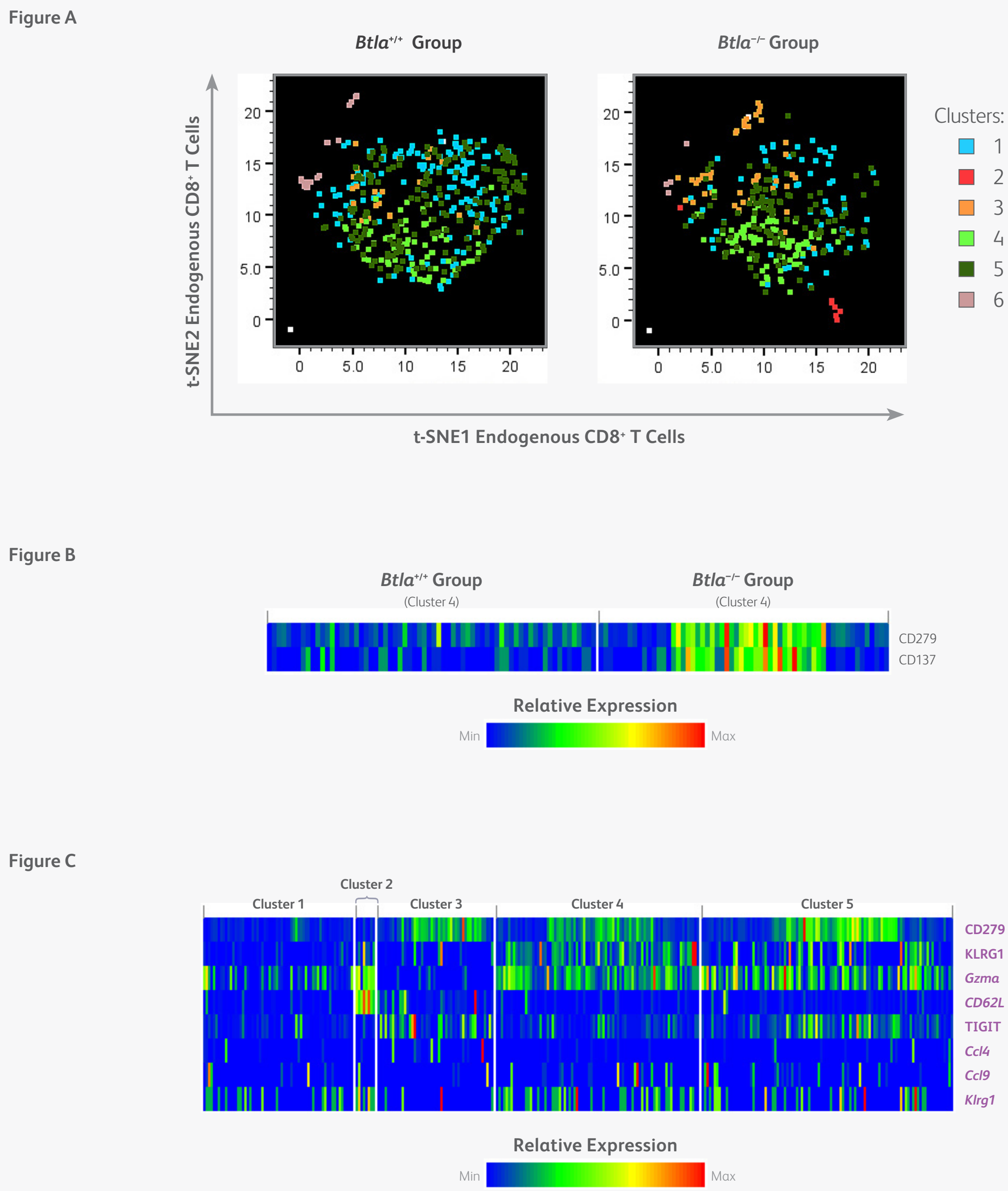
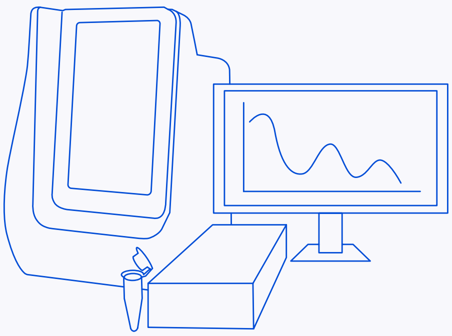


Figure 2. Characterization of endogenous CD8+ T cells in B cell lymphoma-bearing mice. A) Unsupervised clustering of endogenous CD8+ T cells on a t-SNE graph. **B)** Heatmap showing upregulated CD279 and CD137 in a subset of CD8+ T cells from the Btla–/– group compared to the Btla+/+ group. **C)** Heatmap showing a set or proteins and mRNA transcripts that were detected in endogenous CD8+ T cells from the Btla–/– group.

The scientists performed an unbiased clustering analysis using the SeqGeq™ Software plugin for comprehensive characterization of the CD8+ T cells. Overlay of the clusters to a t-SNE plot revealed different clusters (Figure 2A). Differential protein and mRNA analysis showed that a majority of the cluster 4 cells from the Btla–/– group had a distinct signature, with cells co-expressing CD279 and CD137 (Figure 2B). Furthermore, differential expression analysis between the individual clusters in the Btla–/– group revealed the heterogeneity of endogenous CD8+ T cells based on unique patterns of protein and mRNA expression, shown in the representative heatmap in Figure 2C. The researchers observed that endogenous CD8+ T cells displayed signs of activation, expressing proteins like CD137 and Gzma, suggesting involvement in tumor cell elimination.

BD products that enabled this study:



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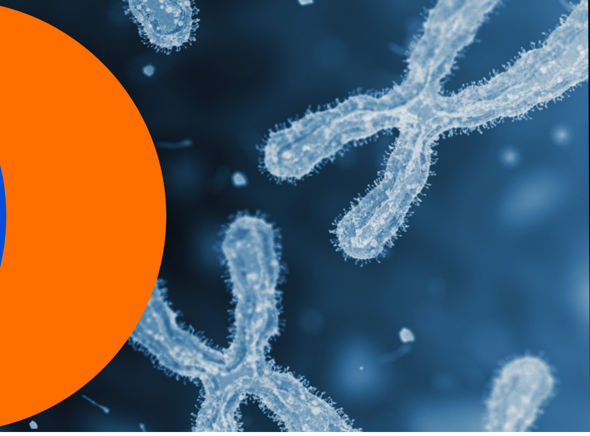

CHAPTER 3

Deciphering the mechanisms of tumor-driven perturbations in hematopoiesis in a mouse melanoma model

The study showcases the effectiveness of an integrated workflow involving cell sorting and single-cell multiomic analysis in understanding the impact of tumors on hematopoiesis. This approach holds promise for uncovering critical insights into the complex cellular dynamics in cancer-related hematopoiesis.

Hematopoiesis is a highly coordinated process in which hematopoietic stem cells give rise to multilineage blood cells through successive series of increasingly lineage-restricted intermediate progenitors. Under steady-state conditions during adult life, hematopoietic stem and progenitor cells (HSPCs) are mainly localized in the bone marrow (BM); however, under pathophysiological conditions such as cancer, extramedullary hematopoiesis can occur in organs like the spleen (SP). This study uses an integrated workflow combining cell sorting and single-cell multiomics to gain insight into the tumor-induced perturbations in hematopoiesis. In it, researchers conducted an in-depth analysis of surface protein and whole transcriptome expression in single hematopoietic cells.

Results

The researchers first used a six-color fluorochrome-labeled antibody panel and the BD FACSMelody™ Cell Sorter to simultaneously isolate four rare HSPC populations: LSK, CMP, CMP and GMP. Next, the researchers sought to determine the immunophenotypic and molecular signature of each of the four sorted populations from healthy BM. To do so, they performed differential expression analyses and found proteins and mRNA transcripts that were upregulated in each of the four populations from healthy BM (Figure 1). Consistent with earlier findings in the literature, several proteins, including CD43, CD49b and mRNA for HLF and FOSB, were upregulated in LSK cells. CMP cells showed upregulation of IRF8, marking myeloid lineage commitment. Also consistent with the literature, CAR1/2and MPO expression enabled discrimination of MEP and GMP-committed cells, respectively.

Find other researchers studying melanoma using the BD Rhapsody™ System technology:

- Johann K, et al. *Nat Commun*. 2021; 12(1):5981.

  Therapeutic melanoma inhibition by local micelle-mediated cyclic nucleotide repression

- Veatch J, et al. *Cancer Cell*. 2022;40(4):393-409.e9.

  Neoantigen-specific CD4+ T cells in human melanoma have diverse differentiation states and correlate with CD8+ T cell, macrophage, and B cell function

- Liu N, et al. *Cell Death Dis*. 2023;14: 170.

  Supplementation with α-ketoglutarate improved the efficacy of anti-PD1 melanoma treatment through epigenetic modulation of PD-L1

In order to gain more insights into tumor-driven perturbations during myelopoiesis, the researchers investigated the differential expression of proteins and mRNA transcripts in CMP cells sorted from the BM of healthy and tumor-burdened mice and the SP of tumor-burdened mice (Figure 2). A number of genes signifying myeloid lineage commitment were upregulated in the tumor-burdened mice. As expected, the differences between the BM of healthy and the BM of tumor-burdened mice were less prominent than the differences between the SP of tumor-burdened mice and the BM of healthy or tumor-burdened mice. Further studies are needed to dissect the functional outcomes and cellular mechanisms associated with these changes.

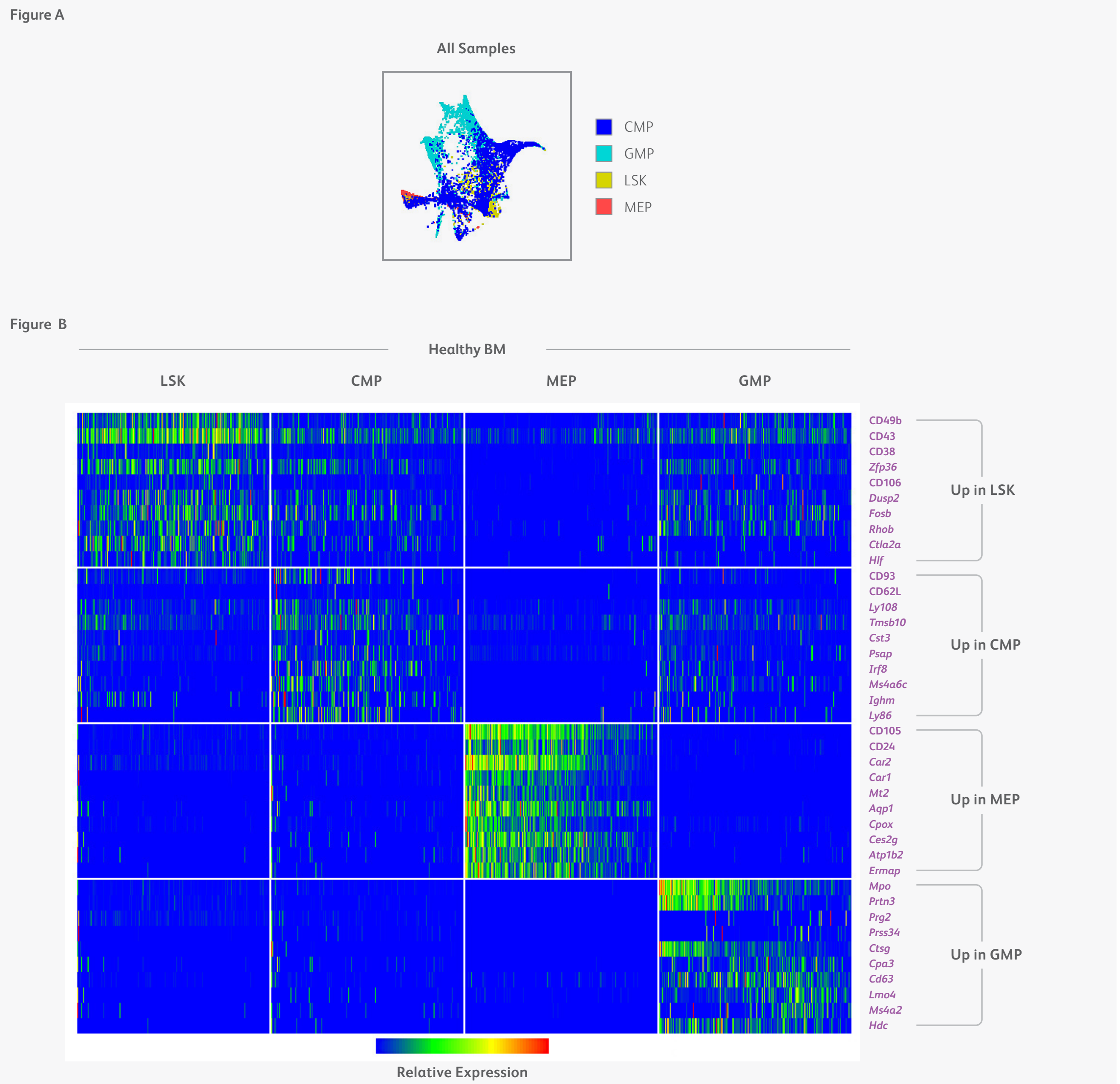

Figure 1. Single-cell protein and whole transcriptome analysis reveal differential expression during normal hematopoiesis. A) A trimap plot showing overlay of LSK, CMP, CMP and GMP populations from healthy BM. **B)** Single-cell heat maps showing the top ten differentially expressed proteins and mRNA transcripts (italic) for each sorted population compared to the remaining populations. A total of 200 representative cells from each population are shown.

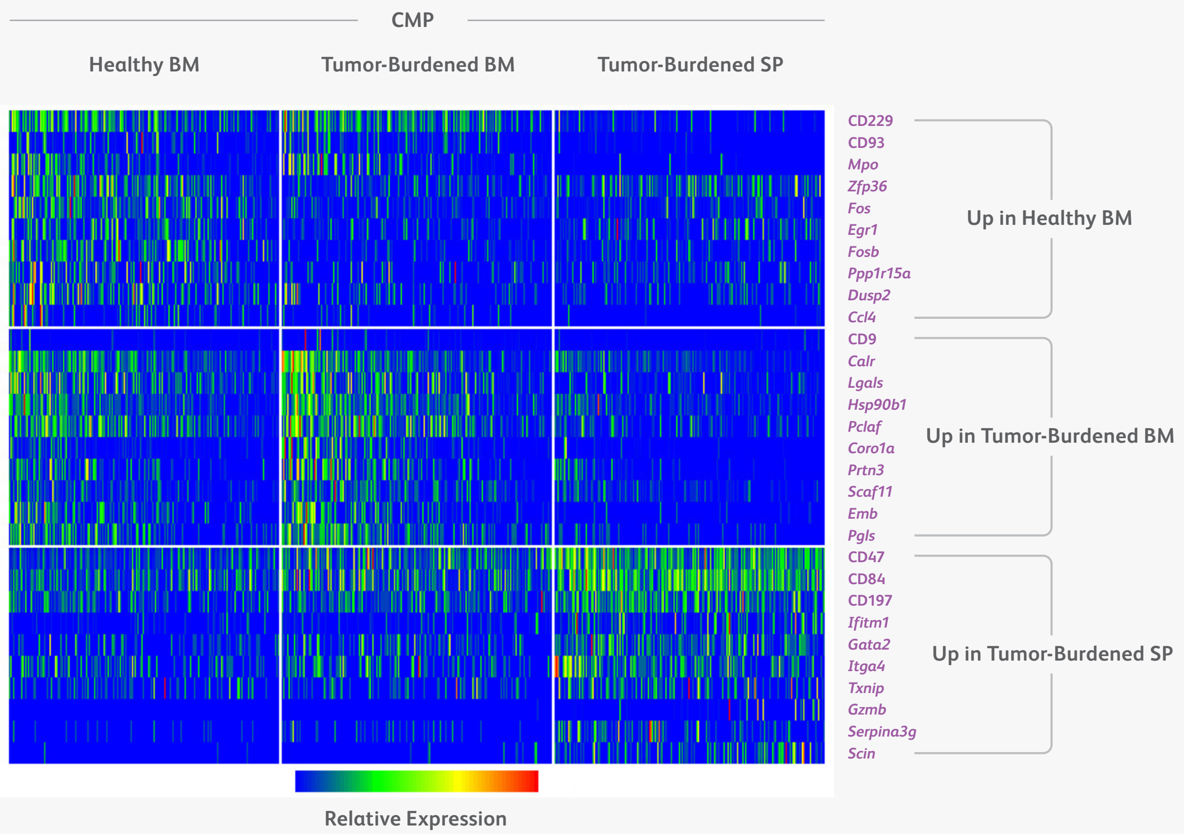

Figure 2. Single-cell and whole transcriptome analyses reveal differential expression during myelopoiesis in healthy and tumor burdened mice. Single-cell heat maps show the top ten upregulated protein and mRNA transcripts (italic) detected in CMP cells from the BM of healthy mice and the BM and SP of tumor-burdened mice compared to the remaining populations. A total of 200 representative cells from each sorted population are shown in the heatmaps.

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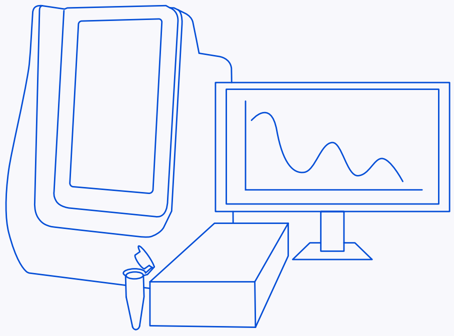

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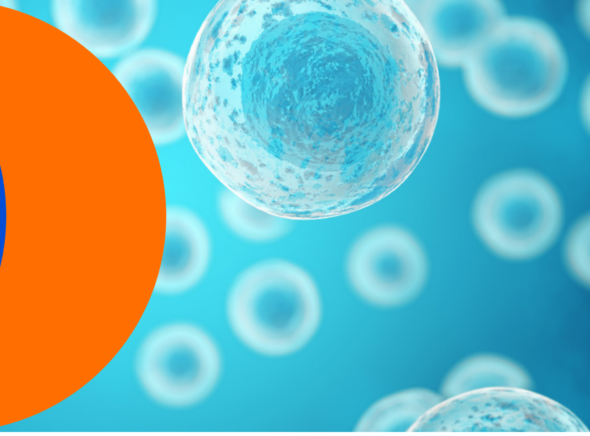


CHAPTER 4

Comprehensive analysis of the T cell repertoire in a mouse colon carcinoma model

In this study, full-length T and B cell receptors were successfully profiled using the full-length BD Rhapsody™ TCR/BCR Multiomic Assay in a mouse tumor model. Comprehensive analysis of the transcriptome, surface protein and TCR repertoire at the single-cell level using the BD Rhapsody™ System enables the investigation of immune cell characteristics and clonotype diversity across multiple samples. The specific TCR sequences expressed by tumor-infiltrating lymphocytes (TILs) identified in this study can provide insights into the recognition and targeting of tumor antigens.

Characterizing T and B cell clonotypes, i.e., populations with unique receptor sequences and hence unique antigen specificities, is instrumental in understanding their role in antitumor activity and the immune system's response in cancer. Studying the full-length TCR and BCR repertoire in the tumor microenvironment enables the identification of potentially therapeutically relevant T cell and B cell clones.

Lymphocytes, made up of T and B cells, typically patrol the body looking for cells that don't belong, such as cancer. As a tumor grows, some T cells recognize the abnormality and become TILs, penetrating a tumor and actively fighting growth from within. Researchers are actively studying how to leverage this function for therapeutic purposes.

Individual TILs can express a diverse variety of variable-joining (VJ) gene combinations that code for cell surface receptors that bind to antigens; however, how the population of individual TILs, each expressing a different type of receptor, mounts a response to recognize and target tumor antigens warrants further study at the single-cell level.

Find other researchers studying colon cancer using the BD Rhapsody™ System technology:

- Li S, et al. *Cell Rep Med*. 2020;1(3): 100039.

  Human tumor-infiltrating MAIT cells display hallmarks of bacterial antigen recognition in colorectal cancer

- Sun R, et al. *Cell & Bioscience*. 2023; 13:62.

  Single-cell transcriptomic analysis of normal and pathological tissues from the same patient uncovers colon cancer progression

- Li S, et al. *Commun Biol*. 2022;5(1): 1326.

  An integrated map of fibroblastic populations in human colon mucosa and cancer tissues

Results

To profile protein and gene expression in T cells and B cells, researchers prepared single-cell suspensions from the healthy mice and those that had been injected with tumor cells representing the left and right femur, spleen, thymus and tumor tissues. All samples were simultaneously stained with fluorescent antibodies, the BD® AbSeq Panel and sample-multiplexing tags. Tissue samples were each stained with the BD® Mouse Single-Cell Multiplexing Kit, while tumor samples were stained with the BD® Flex Single-Cell Multiplexing Kit. Samples were sorted using the BD FACSymphony™ S6 Cell Sorter to separate out T and B cells. To conduct single-cell multiomics experiments, these samples were pooled and loaded into the BD Rhapsody™ Single-Cell Analysis System for analysis. Samples were then prepped for and analyzed with next generation sequencing and data were processed using the BD Rhapsody™ Sequence Analysis Pipeline.

UMAP analysis was performed using AbSeq and mRNA expression on approximately 27,000 high-quality cells (Figure 1). CD3 and CD19 protein expression confirmed the successful sorting of B and T cells.

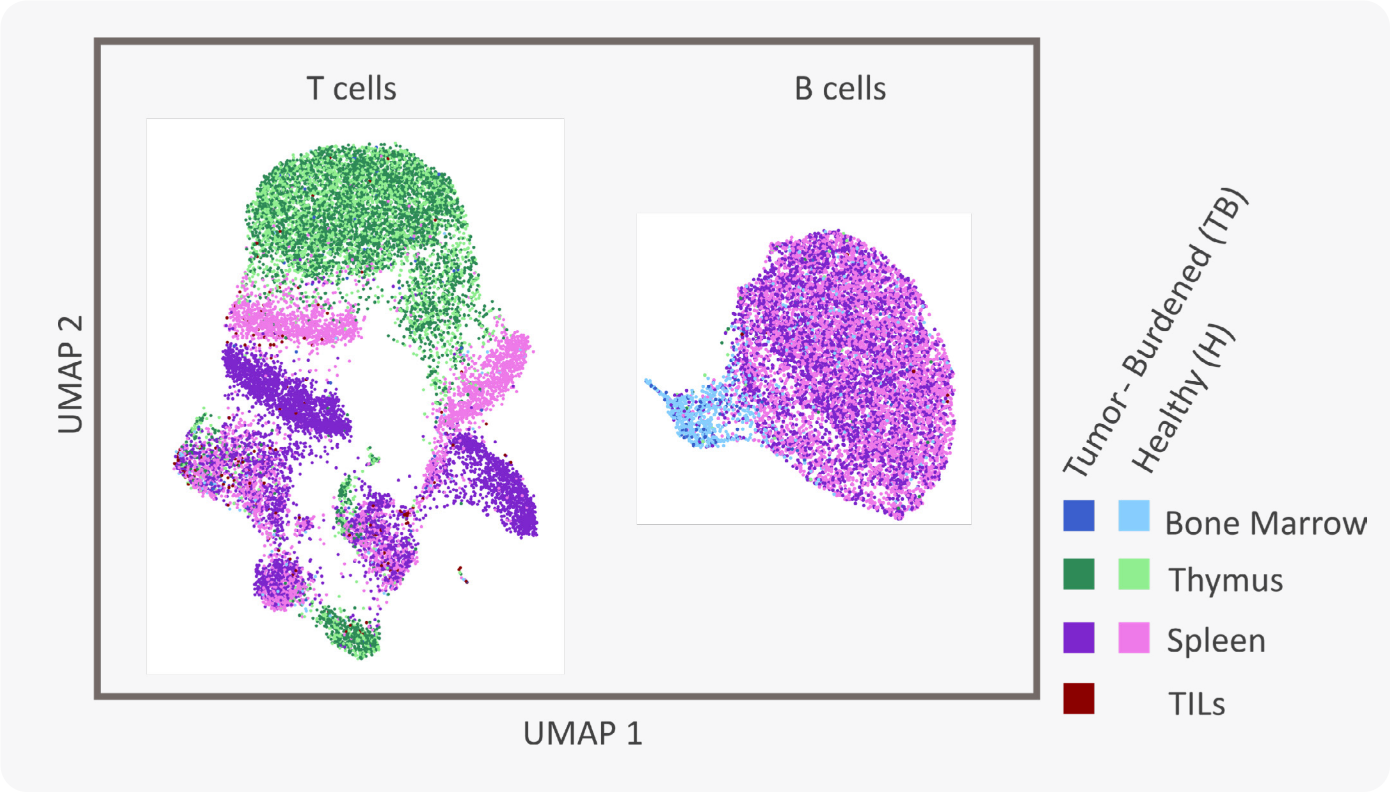


Figure 1. Unsupervised cell clustering across all tissues. UMAP dimension reduction, colors reflecting different tissues.

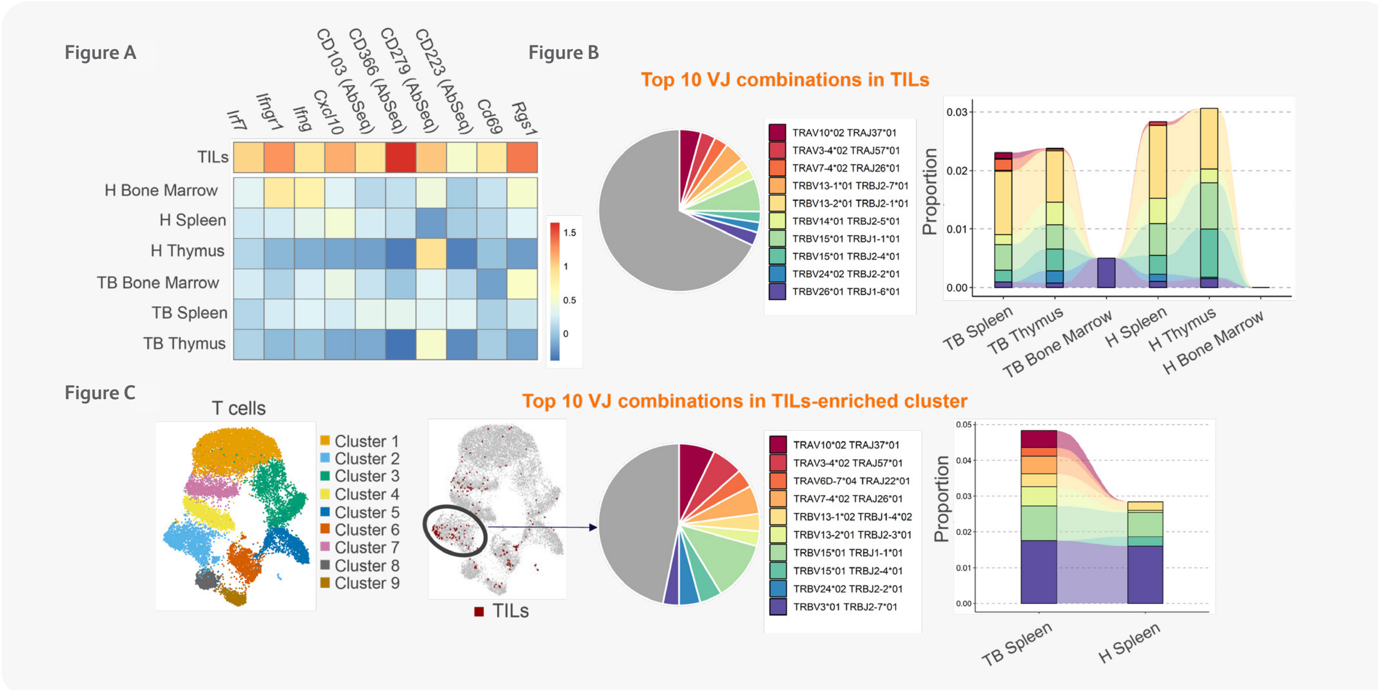
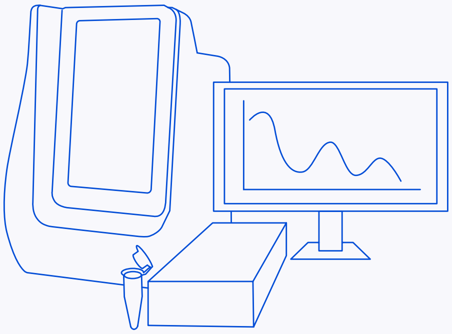


Figure 2. Gene expression, surface protein and single-cell TCR analysis in TILs. A) Heatmap of top differentially expressed mRNAs and proteins in TILs, compared to the T cells from other tissues. **B)** Pie chart shows the distribution of top 10 V and J gene usage in TCR repertoire of TILs, and the bar plot illustrates the frequency of these combinations across other tissues. **C)** Pie chart shows the distribution of the top 10 VJ gene combinations in TCR repertoire of TILs in cluster 2, which has a high frequency of TILs. The bar plot illustrates the frequency of these combinations across tumor-burdened and healthy mice spleen T cells. VJ tracking plots were generated by Immunarch v1.0.0.

To investigate the characteristics of the TILs, differential mRNA and protein expression analysis was performed across different tissues (Figure 2A), which revealed upregulation of T cell activation marker (*Cd69*), tissue-residency (*Rgs1*), and the cytokine and chemokine mRNA (*Ifng* and *Cxcl10*) in TILs. TILs also showed elevated protein expression of T cell exhaustion markers such as CD223 (LAG-3) and CD279 (PD-1). The frequency of the top 10 VJ combinations in TILs were tracked across other tissues (Figure 2B). Zooming in on the TILs cluster (Figure 4C, left), the frequency of the top 10 VJ combinations from this cluster were tracked across the spleen T cells in this cluster. Overall, tumor-burdened mice spleen T cells showed shared TCR combinations with TILs, which were absent in healthy spleen T cells (Figure 2C, right).

BD products that enabled this study:



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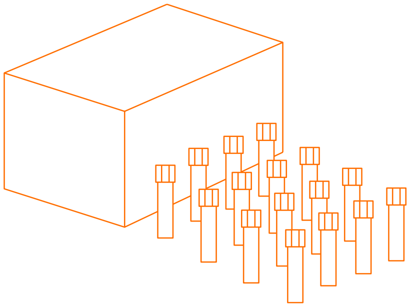

The next generation of BD Rhapsody™ TCR/BCR Multiomic Assays are now available and reveal immune repertoire richness with great sensitivity alongside simultaneous single-cell gene and protein expression analyses. Validated on our new Enhanced Cell Capture Beads V3, BD Rhapsody™ TCR/BCR Next Multiomic Assays enable a highly sensitive analysis of full-length TCR/BCR repertoires for human and mouse and are compatible with cells preserved in the BD® OMICS-Guard Sample Preservation Buffer.

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CHAPTER 5

Deep characterization of tumor-infiltrating leukocytes in a mouse model of pancreatic cancer

This workflow provides valuable insights into the immune composition of the tumor microenvironment, offering potential avenues for the development of novel immune therapeutic strategies for cancer.

The development of cancer immunotherapies is revolutionizing cancer treatment because these supercharge the body's own immune system to fight cancer. However, the effectiveness of cancer immunotherapies varies among patients due to the complex interplay in the tumor microenvironment. T cells, including TILs, are crucial for anti-tumor responses but can be countered by immunosuppressive cells. Single-cell multiomics advances may provide detailed insights into these interactions.

This study employed a comprehensive one-solution workflow involving solid tumor dissociation followed by single-cell multiomic analysis on sorted TILs from mouse pancreatic tumor samples. Marker signatures associated with B cells, T cells and myeloid cells were identified, revealing distinct subclusters within the tumor microenvironment.

Results

A mouse pancreatic tumor was divided into four pieces and dissociated by four different methods. Dissociated cells were co-stained with a BD® AbSeq Antibody-Oligo Conjugate Panel, BD® Mouse Immune Single-Cell Multiplexing Kit, live/dead marker and fluorescent CD45. A 12-color flow cytometry panel was designed to identify tumor cells and tumor-infiltrating leukocytes. Live CD45+ cells were sorted from each sample using the BD FACSMelody™ Cell Sorter and pooled together for single-cell capture using the BD Rhapsody™ Single-Cell Analysis System.

All leukocytes acquired from different tumor dissociation methods were combined. UMAP dimensional analysis was performed using all cells. Distinct clusters were observed on UMAP. Using cell-type specific markers, the researchers differentiated major cell types in the tumor microenvironment, including B cells, myeloid cells and T cells (Figure 1A).

Find other researchers studying pancreatic cancer using the BD Rhapsody™ System technology:

- Wang L, et al. *Gut*. 2023;72(5):958-971.

  Single-cell RNA-seq analysis reveals BHLHE40-driven pro-tumour neutrophils with hyperactivated glycolysis in pancreatic tumour microenvironment

- Mao X, et al. *Genes*. 2022;13(6):1015.

  Single-cell transcriptomic analysis of the mouse pancreas: characteristic features of pancreatic ductal cells in chronic pancreatitis

- Xue M, et al. *Nature Communications*. 2023;14:4600.

  Schwann cells regulate tumor cells and cancer-associated fibroblasts in the pancreatic ductal adenocarcinoma microenvironment

Differential marker expression analysis revealed the unique marker signatures associated with each cell type (Figure 1B). Next, they performed an in-depth analysis of the immune cells in the tumor microenvironment. Using the Seurat pipeline, they identified 10 different cell clusters within the tumor microenvironment and uncovered cluster-specific marker signatures (Figure 1C). With this data, the scientists were able to identify different T cell subpopulations with different functional statuses, such as CTLA4-expressing T cells or cytotoxic T cells (Figure 1C). Overall, the analysis enabled by a single-cell target gene panel combined with the BD® AbSeq Assay revealed specific details about tumor heterogeneity in the pancreatic tumor mouse model.

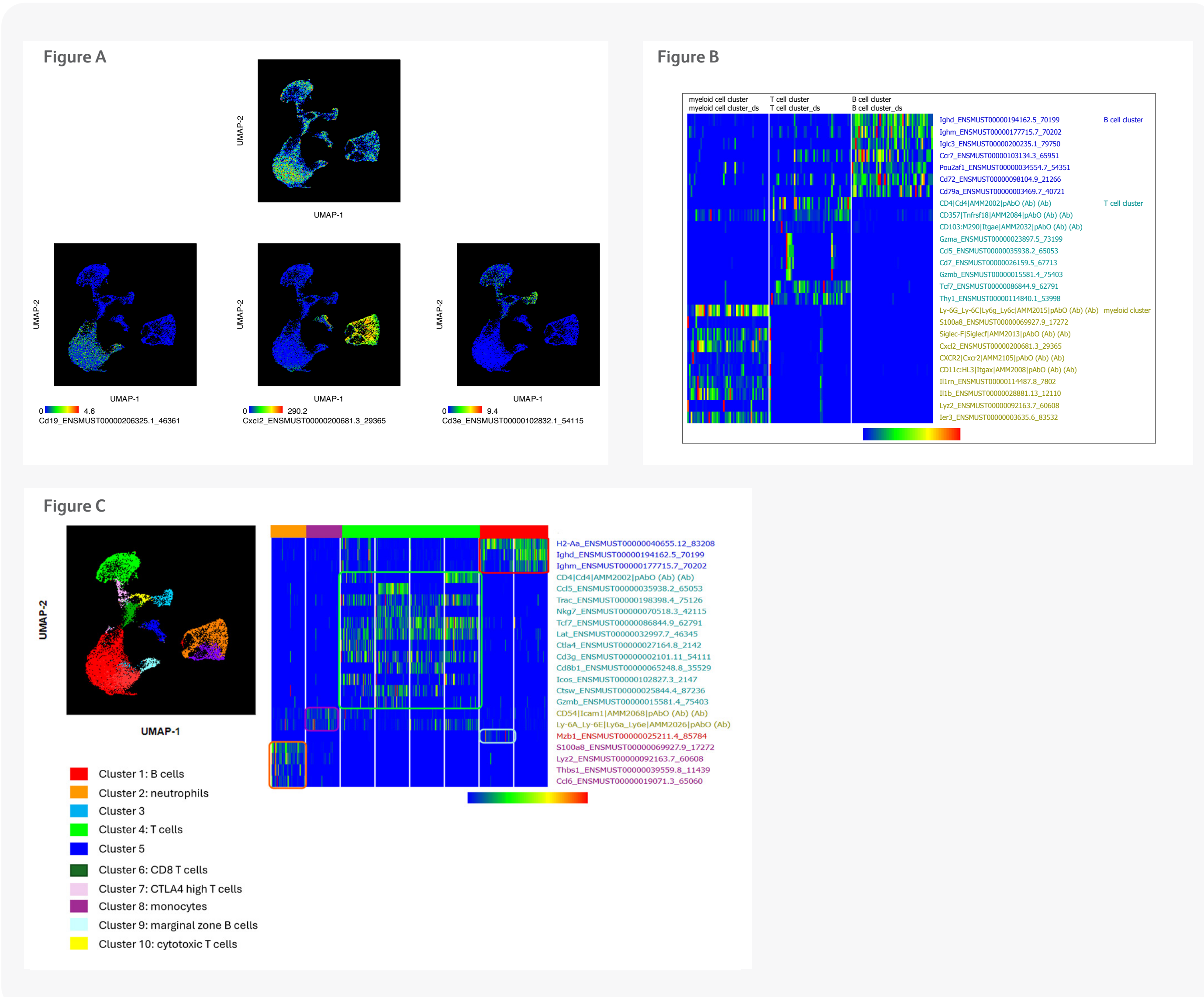
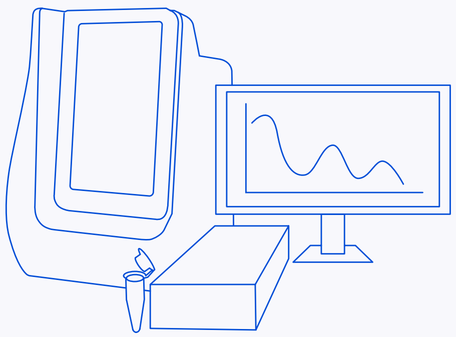


Figure 1. In-depth characterization of tumor-infiltrating leukocytes using single-cell multiomic analysis. A) Dimensional reduction by UMAP and expression of key cell type markers. UMAP was performed using a targeted gene panel including around 400 genes and all AbSeq markers. B cell marker CD19, myeloid gene marker CXCL2, and T cell-associated gene CD3D were used to differentiate T cell, B cell, and myeloid cells. **B)** Identification of marker signature of B cells, T cells and myeloid cells. **C)** Seurat analysis reveals 10 different clusters as coded by different colors (left). The marker signature associated with each cluster was identified, revealing different immune cell subsets infiltrated in the tumor microenvironment (right).

BD products that enabled this study:



- BD fluorochrome antibodies
- BD® AbSeq Antibodies
- BD® SMK
- BD Rhapsody™ Immune Response Targeted Panel (Mouse)
- BD Horizon™ Tumor & Tissue Dissociation Reagent
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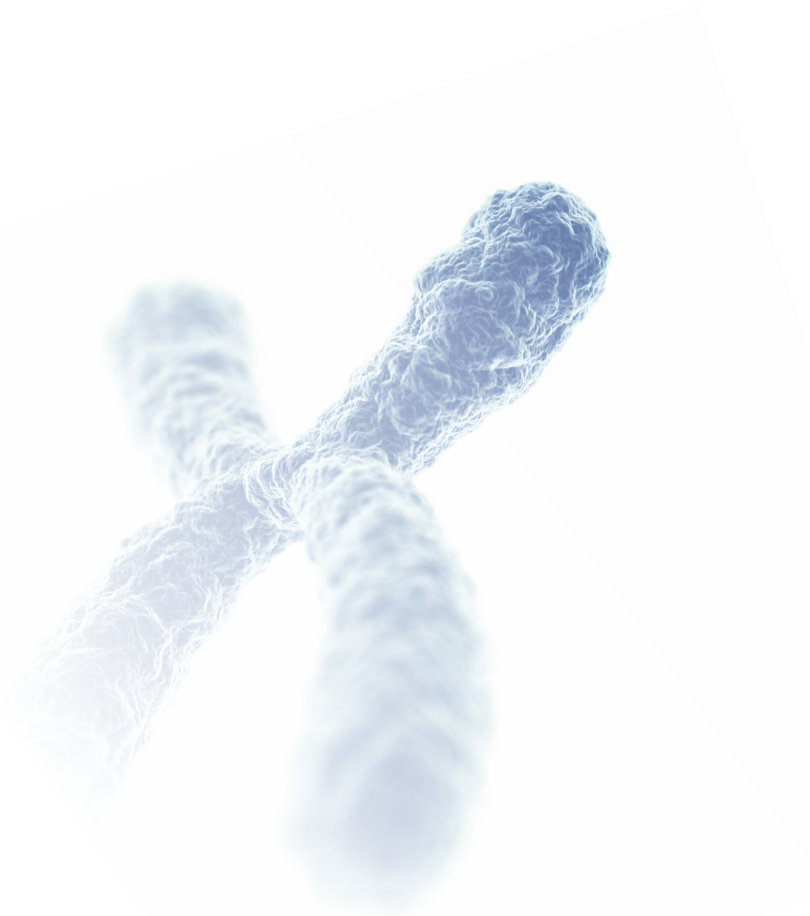

Discussion

The field of immuno-oncology is evolving rapidly, bringing with it a plethora of clinical research. Exciting new approaches are being explored, including immune checkpoint inhibitors and newer modalities such as CAR T cell therapies that actively target tumor tissue and antibody therapies that engage immune cells to produce an anti-tumor response. These strategies leverage immune system activation in the tumor microenvironment to recharge the body's natural defenses, restoring its ability to detect and fight virtually any type of cancer while sparing healthy cells. The result: more targeted compounds under investigation with the potential to cause fewer side effects.

In the field of immuno-oncology, there is a growing need for single-cell multiomics due in large part to the heterogeneity of cells present in the tumor microenvironment. The comprehensive workflow demonstrated by the five chapters here demonstrates how BD instruments can generate crucial single-cell, multiomic data that inform our knowledge of the vast array of immune cells present in the TME and how they respond to their surroundings. These data may inform the next generation of immunotherapies, for example, the use of TILs to combat cancer. Crucially, the BD Rhapsody™ System workflow allows users to isolate and interrogate a broad range of cell types, including those that are rare or fragile, thereby capturing a more complete picture of the immune system's response to cancer.

With the right tools to gather and unravel multiomic data, researchers can dive deeper into the cellular landscape of the immune response to cancer, identifying new challenges and opportunities. By dissolving obscurity through comprehensive single-cell multiomic experiments, the field can continue pursuing promising new research avenues to uncover new ways to combat cancer.

Partnering to advance science

The BD Rhapsody™ Single-Cell Analysis System offers integrated services and support to match the quality of our proven single-cell multiomics technology. When you choose our products, it's the beginning of a relationship. BD is committed to supporting the breadth of applications of single-cell multiomics offerings. Learn how BD single-cell multiomics products can help you further your immuno-oncology research by reaching out to us at **scomix@bdscomix.bd.com**.

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