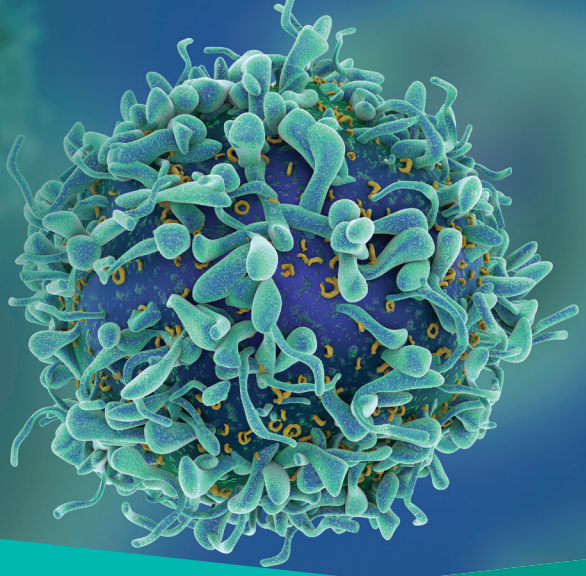
BD Researcher's Digest
Vol. 2 | January, 2020

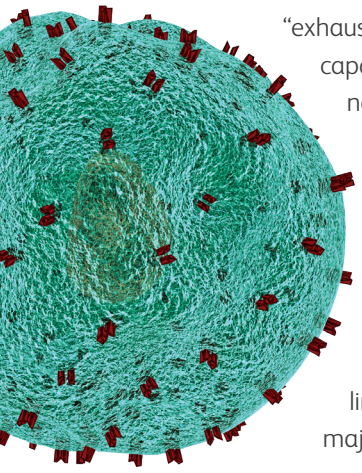


I. SPOTLIGHT ARTICLE

# T cell Exhaustion: What Does it Mean?

*Contributed by:*
Ravi Hingorani, PhD | Immunology enthusiast, BD Biosciences Senior Technical Service Scientist

T cells encounter antigens to start an intrinsic pathway that drive differentiation and clonal expansion for a functional response. Subsequently, when the antigen source is eliminated, the effector T cell undergoes apoptosis, with a few surviving to become effector memory T cells. In chronic infections and cancer, T cells are exposed to persistent antigen and/or inflammatory signals. This scenario is often associated with deterioration of T cell function: a state called "exhaustion".[1,2] Exhausted T cells have reduced capacity to respond to stimulation, and do not actively proliferate. They can arise as a result of chronic stimulation such as LCMV, and responses to the tumors in the tumor microenvironment. Though associated with hypo-functional state, exhausted T cells in response to chronic stimulation are seen to persist for a long time and partially contains lingering infections without causing major immunopathology.[3]



Functional traits of hypo-responsiveness in the tumor microenvironment is thought to be due to inadequate priming of T cells leading to a "dysfunctional" state.[4] Epigenetic studies show distinct differentiation states during tumor progression. With continued tumor-specific encounters, the chromatin changes from the initial state of being therapeutically reprogrammable to a plastic dysfunctional state that cannot be rescued.

Additionally, compared to effector or memory cells, exhausted T cells in the tumor microenvironment tend to express multiple inhibitory receptors such as **PD1**, **TIM3**, **LAG3**, **CTLA4**, **TIGIT**, **CD38**, **CD39**, **2B4**, and **CD101**[1,5] indicating a modulation through immune check-point regulatory pathways. Understanding this state, along with the extrinsic and cell-intrinsic pathways responsible for non-responsiveness, is a major field of research for tumor immunotherapy. Issues in treatment revolve around how to invigorate the exhausted cells and also around how to keep the altered responsive state permanent.[5]

Understanding the molecules and mechanisms involved in T cell "exhaustion" has been made easier in using a high parameter

approach. This approach helps increase the potential quality and impact of data, by allowing for analysis beyond two-dimensional gates. Machine learning and automatic data clustering can identify as-yet-unknown populations in data. The recent Optimized Multicolor Immunophenotying Panel (OMIP) using the BD FACS Symphony A5 by Giles and Chattopadhyay wherein, researchers used a 28-color or 30-parameter approach to comprehensively characterize cells expressing check point markers.[6]


**References**

1. Philip, M. & Schietinger, A. Heterogeneity and fate choice: T cell exhaustion in cancer and chronic infections. *Curr. Opin. Immunol.* 58, 98–103 (2019).
2. Wherry
3. McLane, L. M., Abdel- Hakeem, M. S. & Wherry, E. J. CD8 T cell exhaustion during chronic viral infection and cancer. *Annu. Rev. Immunol.* 37, 457–495 (2019).
4. Philip, M., Fairchild, L., Sun, L., Horste, E.L., Camara, S., Shakiba, M., Scott, A. C., Viale, A., Lauer, P., Merghoub, T., Hellmann, M. D., Wolchok, J. D., Leslie, C. S., and Schietinger, A. Chromatin states define tumour-specific T cell dysfunction and reprogramming. *Nature.* 545, 452–456 (2017).
5. Blank, C.U., Haining, W.N., Held, W., Hogan, P.G., Kallies, A., Lugli, E., Lynn, R.C., Philip, M., Rao, A., Restifo, N.P., Schietinger, A., Schumacher, T.N., Schwartzberg, P.L., Sharpe, A.H., Speiser, D.E., Wherry, E.J., Youngblood, B.A., and Zehn, D. Defining 'T cell exhaustion'. Nature Reviews Immunology 19, 665-674 (2019).
6. Nettey, Giles, and Chattopadhyay, P: OMIP-050: A 28-color/30-parameter Fluorescence Flow Cytometry Panel to Enumerate and Characterize Cells Expressing a Wide Array of Immune Checkpoint Molecules. *Cytometry A*, 2018

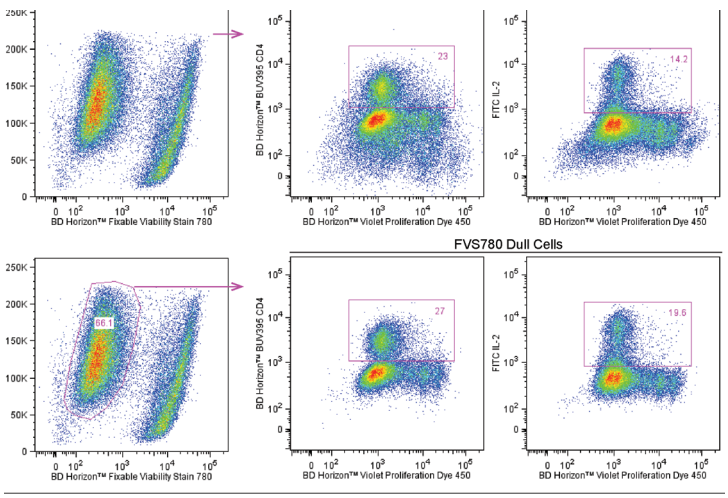

## II. TIPS AND TRICKS

# Why Should We Perform Live Dead Cell Discrimination While Performing Multicolor Experiments?


*Contributed by:*
Stuart Williams, PhD. | BD Applications Support Team Manager and Science Aficionado


Using viability dyes/stains to distinguish live-dead cells is a critical part of multicolor experimental design as one is able to exclude dead cells in the stained samples. It is important to gate our dead cells as they can bind non-specifically, and can lead to incorrect data and conclusions. Viability dyes used for flow cytometry are membrane exclusion dyes.

The traditional dyes (PI, 7-AAD) can only be used with fresh samples, as fix or perm protocols destroy the cell membrane integrity and the cells would stain as if they were dead. To address this gap, BD developed a new family of viability dyes excited across different laser lines based on dye exclusion. They are also compatible with intracellular staining protocols meaning they work well on fixed cells. Introducing the **BD Horizon™ Fixable Viability Stains (FVS).**




The basic principle behind the BD Horizon™ FVS dyes is that live cells have an intact cell membrane and the dye is unable to enter the cell and stain the cell. Cells that are in late stages of apoptosis or are dead no longer have an intact cell membrane, and the dyes can enter the cells, bind and stain the cells. When run on a flow cytometer, the dead cells show a strong signal in the viability dye channel, allowing them to be unambiguously identified. Since only dim or negative cells are analyzed, there is little spread of signal in neighboring channels from the viability dye. Samples are first stained with these dyes in a protein free buffer, then stained as usual. Because of this workflow, these dyes can be used with fix or perm protocols to accurately identify live or dead cells.

## RESOURCES

**Studying T cell Exhaustion using a Single-Cell Multiomic approach**

**Webinar - Nature**

- Topic: Single-Cell Multiomic Analysis of T cell Exhaustion
- Webinars Page

- Exploring Tumor Heterogeneity using the BD FACSMelody™ Cell Sorter using a Single Cell Multiomic approach
- Using the BD FACSMelody for yeast cell sorting

## III. SERVICE CORNER


As previously communicated, Microsoft® will no longer support Microsoft Windows® 7 operating system (OS) beginning January 14, 2020. In response to this announcement, BD plans to release versions of BD FACSDiva™ software, BD FACSCanto™ clinical software, and BD FACS™ SPA software that are compatible with Microsoft Windows 10 Operating System (OS). BD plans to release the aforementioned software versions by Dec.'19.

BD Field Service Engineers are required to perform the upgrade. BD application support is also available for customers with a variety of instruments, templates, or complex workflows. Please note it is your responsibility to back up all data, templates, and necessary reports prior to the upgrade. As you upgrade your workstations to Microsoft Windows 10 OS, the only legacy workstations eligible for an upgrade are the HP 2240 workstations. Please note all other legacy workstations will be replaced with a new workstation using Microsoft Windows 10 OS.

BD FACSDiva™ v9 is compatible with Windows 10 OS and will support all versions of the BD FACSCanto™, BD FACSAria™ II, BD FACSAria™ III and BD FACSAria™ Fusion, BD® LSR II and BD LSRFortessa™, and BD FACSCelesta™ instruments, including SORP versions of the aforementioned instruments. BD FACSCanto™ clinical software v4 and BD FACS SPA software v6 will be available at the same time. **If you would like to contact a BD support representative for details on pricing for upgrading your workstation,** click **here**.

## IV. SOFTWARE UPDATES

- The BatchLR plugin released for SeqGeq™ can help correct for batch effects that can be an issue for combined datasets from single-cell RNA sequencing experiments. The tool makes use of the popular MNN algorithm developed as an R package – "batchelor" – by Aaron Lun, out of the Sanger Institute's Marioni Lab. (for more information, read their **paper** in Nature Biotech)
- Another powerful new plugin available for both FlowJo and SeqGeq™ provides an entire pipeline for analysis, including dimensionality reduction, clustering, differential expression analysis, as well as a new interactive 3D plotting tool based on the **iCellR** package developed by Alireza "Reza" Khodadadi-Jamayran, a brilliant bioinformatician at New York University School of Medicine.
- The SeqGeq™ bioinformatics platform itself has received a **major update**, bringing new high-resolution plots and support aimed at bulk sequencing analysis, such as mouse-over tool tip information within dot plots of both genes and cells/samples.

## V. MEET TEAM BD

**Shilpi Verma, PhD | BD Senior Scientist, Development Tell us a little about yourself.**

I bring immunology expertise to my role as a Senior Scientist on the New Content Team. New antibody content development is special to me as it allows me to be at the forefront of immunology research, understanding what new tools we can help create to answer the bigger scientific questions.

Prior to BD, I trained as cellular immunologist & virologist with emphasis on mouse immunology and TNF family members, with Dr. Carl Ware and Dr. Chris Benedict at the La Jolla Institute for Immunology.

**What has been a special project you've been involved in at BD that has enhanced the customer workflow?**

Every new reagent that we release brings the customer one step closer to accurately identifying and studying the cell populations and their interactions with each other. It helps them make novel, meaningful discoveries relevant to the field of healthcare. A couple of reagents that we released this past year that are relevant to current research in immunotherapy and immune

## V. MEET TEAM BD CONTINUED

exhaustion are: **Anti-Eomes Antibody** (clone X4-83 – a unique clone that has the ability to recognize both mouse and human Eomes and Anti-mouse CD279 (PD-1) (**clone J43**) which we released in a format that would be compatible for functional studies like blocking ligand binding in no azide low endotoxin format.

### What are some hobbies that you enjoy in your spare time?

I love reading, hanging out with friends, doing fun crafts and playing with my kids.

### Your favorite quote:

"Arise, awake, and stop not till the goal is reached"
*– Swami Vivekanand*

## VI. NEW REAGENT RELEASES

As a company committed to bringing the best tools to answer the biggest scientific questions, we recently released a new monoclonal antibody for CCR7 (**CD 197**) by flow cytometry. The new clone 2-L1-A has superior staining characteristics as compared to our existing product.

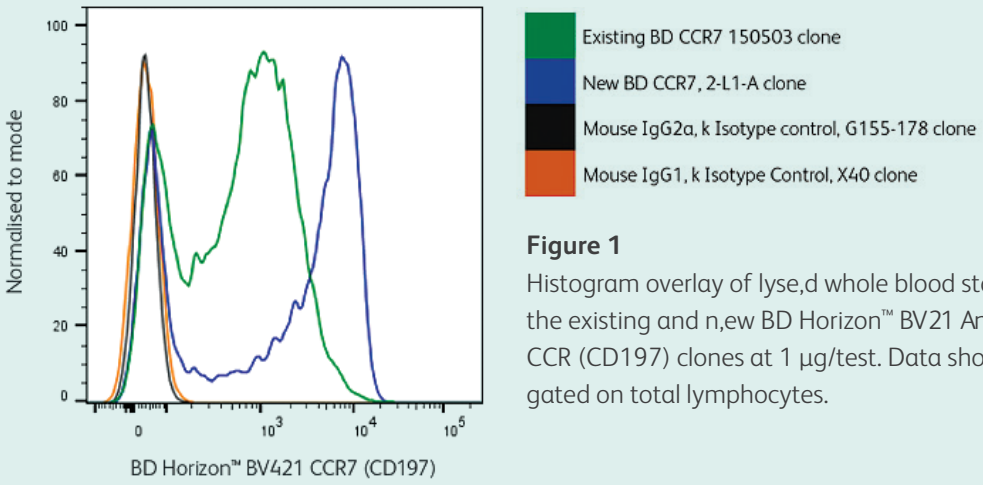



**Figure 1**
Histogram overlay of lyse,d whole blood stained with the existing and n,ew BD Horizon™ BV21 Anti-Human CCR (CD197) clones at 1 µg/test. Data shown is gated on total lymphocytes.

Shop our new CCR7 (CD 197) 2-L1-A antibody clone to ease your panel design. Click **here** to view all available formats.

# Thanks for reading this issue of BD Researcher's Digest

If you have any questions for our contributors, would like to suggest topics for our next issue, or need technical support, contact our Research Applications Team. Please email **researchapplications@bd.com** or call **1-877-232-8995** option 2; Option 2.

Follow us!