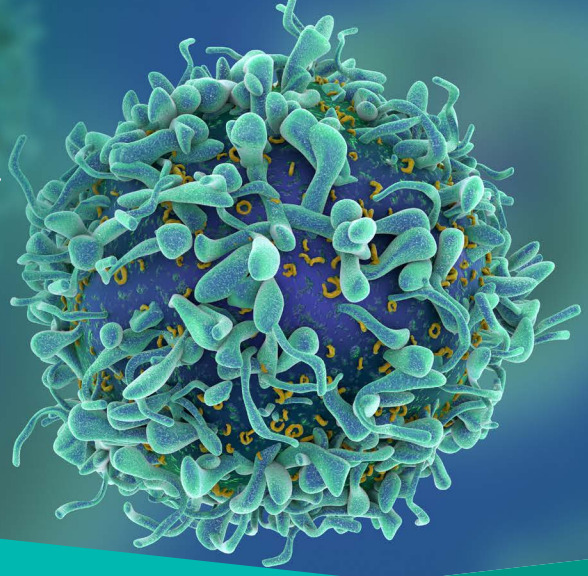
BD Researcher's Digest
Vol. 1 | November, 2019



I. SPOTLIGHT ARTICLE

Regulatory T Cells And Their Role In Therapeutic Efficacy

Contributed by:
Ravi Hingorani, PhD | Immunology Enthusiast, Senior Scientific, and Applications Support

Studies on regulatory T cells (Treg) in preventing autoimmunity and other disorders has led to identification of different subsets within the Treg population. Current classification of Tregs describe them as a mixed population that contains thymus derived Treg (tTreg) comprising of naïve Treg (nTreg) and effector Treg (eTreg); peripheral *invivo* activated Treg (pTreg); and a small population of functionally unstable Treg population that can lose Foxp3 expression and become "ex-Tregs". Each of these has been defined using knock-out mice, thymectomized mice, and GFP mice, to identify and characterize them. Intracellular Forkhead box protein 3 (Foxp3) and Helios, tend to be the major markers used to define Tregs along with surface **CD15**, **CD25**, **CD39**, **CD45RA**, **CD73**, **CD86**, **CD152**, **CD223**, **CD304**, and **LAP**. While intracellular transcription factors (**Foxp3**, **Helios**) are limiting in their use for Treg isolation; many of the surface markers are also be expressed by conventional cells making Treg identification challenging.

The Foxp3 transcription factor is considered most definitive for identification of Treg cells when it was found that this gene is necessary and sufficient for suppressive activity. Surprisingly, after all these years of study, we still do not know the exact *invivo* mode of Treg suppression. Treg suppression is completely antigen non-specific, once Tregs themselves get activated through their T-cell receptor giving rise to inhibition of a diverse T-effector population. Development of therapeutic manipulation of Treg function in humans is still an open field for its use in a given disease state. Given that Tegs have been shown to play a significant role in development of resistance to immune check point inhibitors that leads to tumor relapse/ tolerance, it is important to understand how to modulate them for therapeutic efficacy. Pre-Clinical trials have shown that depletion of Tregs at tumor sites is more important than activation of Teffector cells. On the other hand, studies in mice show that Treg play a central role in Type I diabetes (T1D) by controlling autoreactive cells and failure or instability of Treg contribute to development of the T1D. Studies on homeostasis and balance of Treg has led to better management of tissue transplantation, autoimmunity, and anti-tumor responses.

References

1. Shevach, E. Foxp3+T regulatory Cells: Still many unanswered questions—A perspective after 20 years of study. Fron Imm 2018; Published online DOI: 10.3389/fimmu.2018.01048.
2. Romano, M., Fanelli, G., Albany, J.C., Giganti, G., and Lombardi, G. Past, present, and future of regulatory T cell therapy in translation and autoimmunity. *Front. Imm.* 2019, DOI https://doi.org/10.3389/fimmu.2019.00043.
3. Saleh, R., Elkord, E. Cancer Letters 2019, DOI https://doi.org/10.1016/j.canlet.2019.05.003
4. Simpson, T.R., Li, F., Montalvo-Ortiz, M., Sepulveda, M.A., Bergerhoff, K., Arce, F., Roddie, C., Henry, J.Y., Yagita, H., Wolchok, J.D., Peggs, K.S., Ravetch, J.V., Allison, J.P., and Qezada, S.A. Fc-dependent depletion of tumor-infiltration regulatory T cells co-defines the efficacy of anti-CTLA-4 therapy against melanoma. *J. Exp Med* 2013. DOI 10.1084/jem.20130579
5. Sharma, A., and Rudra, D. Emerging functions of regulatory T cells in tissue Homeostasis. *Front Immunol* 2018 DOI: 10.3389/fimmun.2018.00883.

BD Resources for T Cells

- T Cell Research
- Datasheet: Sorting and Downstream Functional Assessment of Regulatory T Cells

II. TIPS AND TRICKS

How to Protect Your Sort Against Side Stream Instability In 4-Way Sorts

Contributed by:
Max Darch Ph.D. | Technical Support Scientist
Shelley Johnson | Product Course Developer and Flow Cytometry Sorting Buffs

As a guideline, operators can assign high-abundance populations (relative to the others being sorted) to the interior collection tubes of a 4-way sort as a means to lower the impact of cross-contamination. This will assist in minimizing the frequency of sorted droplets passing over non-target tubes and help protect against cross-contamination in situations of stream instability. In cases of stream instability, the interior tubes would have the greatest potential for contamination impact. However, if stream stability is problematic, it is more important to address the underlying cause. For example, a cause of side stream instability may be a result of cell size. As cell size approaches the size of a sort nozzle the shape of droplets containing a target event will be affected, and that can lead to deflection trajectory issues. In this scenario, it is important to consider nozzle size, and move up in nozzle size when possible to increase the drop size relative to the sorted particle. This will lower the effect the particle has on altering droplet morphology, leading to tighter and more uniform side-streams. When uncorrected, you will typically find a higher impact of cross-contamination in the interior collection tubes. Additionally, it is important to use a 4-way purity sort mode in a 4-way sort as this will minimize deflection trajectory issues of adjacent, similarly charged, particles by always performing a 1-drop sort.

III. SINGLE CELL MULTIOMICS

Gain deeper knowledge at single cell level using BD Single Cell Multiomics Systems that also help lower your sequencing costs together with BD® AbSeQ antibody-oligonucleotide conjugates.

Learn more >

IV. SOFTWARE UPDATES

Contributed by:
Ian Taylor Ph.D. | Fan of Flow Cytometry Analysis simplified

FLOWJO

FlowJo™ software provides a user-friendly bioinformatics platform for flow cytometry data analysis as well as SeqGeq v1.6.0™ for single cell sequencing data analysis.

New updates to FlowJo v10.6.1:

- With newly-added **BD FACSDiva™** support, users can now import and export gates directly to and from their BD FACSDiva experiment.
- **Platform overlays** unlock the ability to overlay kinetic, proliferation, and cell-cycle plots from one sample onto another or multiple samples.

IV. SOFTWARE UPDATES CONTINUED

New updates to SeqGeq v1.6.0:

- Create high-resolution figures, making plots look and feel more immersive and informative.
- Change dot sizes and shapes individually within overlays, for those hard to spot rare cell populations.
- Mouse-over annotations within dot plots, so you immediately know which cells are which within a CellView plot, or to identify the genes of interest in GeneView at a glance.

New plugins available on FlowJo Exchange add additional functionality including:

- BatchLR – helps remove the notorious and elusive batch effects associated with different single cell RNA sequencing runs. (for SeqGeq only)
- DankRootCellR – runs an entire pipeline of advanced analyses options, including:
  - dimensionality reduction
  - unsupervised clustering
  - differential expression analysis
  - interactive 3D graphs

(available for both FlowJo and SeqGeq)

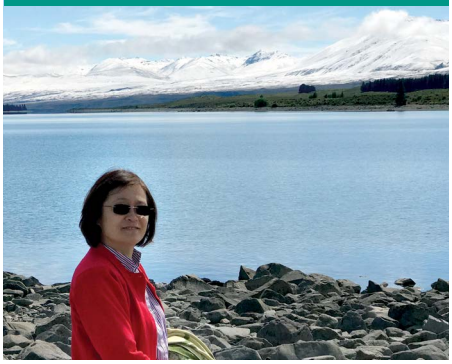
Learn more >

V. SERVICE CORNER

Have you heard of BD Assurity Linc™ Software Security? The BD Assurity Linc connection enables BD to monitor your instruments and provide you with fast and efficient service and support.

Whitepaper: BD Assurity Linc™ Software Security

Meet Li Li!



Your favorite quote:

"The secret of success is to do the common thing uncommonly well."
-John D. Rockefeller Jr.

VI. MEET TEAM BD: LI LI

Tell us a little about yourself.

"My name is Li Li, I received my M.D. from Peking Union Medical College (PUMC) at Beijing China, and Ph.D. in Immunology from Dr. Max Cooper from University of Alabama at Birmingham (UAB), a recent recipient of 2019 Albert Lasker Basic Medical Research Award together with Dr. Jacques Miller for their identification of two distinct classes of lymphocytes, B and T cells respectively.

Currently, am managing the New Content Ab development wherein, we develop broad portfolio of high quality mAbs that enable researchers to identify, isolate and analyze cell of interest towards deeper cutting-edge discovery and understanding disease."

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

"The project that stands out in my mind, was a technology that we developed in collaboration with Dr. Gary Nolan's laboratory in Stanford wherein, flow cytometry based approach is used to detect and analyze heterogeneous signaling responses in primary cells that, are now available as BD Phosflow TM products. Fluorescent antibodies specific for cell surface markers added together with the BD Phosflow reagents further helps to identify cell subsets involved in signaling. Additionally, as a part of this project we also developed helped appropriate buffers and performed compatibility analysis of the surface reagents with these buffers to help design multiple color experiments. All of the **data** is available in the resource page on our website as tools."

https://www.bdbiosciences.com/en-us/applications/research-applications/intracellular-flow

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

I enjoy traveling around the world and visiting national parks. The best place visited including Alaska and New Zealand.

VII. NEW REAGENT RELEASES

BUV 615 is the newest addition to the family of BD Horizon Brilliant™ Ultraviolet Reagents, providing ease and flexibility with panel design. This dye is a tandem fluorochrome with an excitation maximum near 350 nm and an emission maximum near 615 nm. BD Horizon Brilliant BUV615 can be excited by the ultraviolet laser 355 nm and detected with a 610/20 filter (similar to PE-CF594) and a 595 nm. Some of the BUV 615 conjugates available from us are highlighted below:

Anti-Human Antibodies

Description	Clone	Apps	Reg	Format	Size	Cat No.
Hu CD4 BUV615 SK3 100Tst	SK3	FCM	RUO	BUV615	100 Tests	612987
Hu CD4 BUV615 SK3 25Tst	SK3	FCM	RUO	BUV615	25 Tests	612988
Hu CD19 BUV615 SJ25C1 100Tst	SJ25C1	FCM	RUO	BUV615	100 Tests	612989
Hu CD19 BUV615 SJ25C1 25Tst	SJ25C1	FCM	RUO	BUV615	25 Tests	612990
Hu CD279 (PD-1) BUV615 EH12.1 100Tst	EH12.1	FCM	RUO	BUV615	100 Tests	612991
Hu CD3 BUV615 UCHT1 100Tst	UCHT1	FCM	RUO	BUV615	100 Tests	612992
Hu CD3 BUV615 UCHT1 25Tst	UCHT1	FCM	RUO	BUV615	25 Tests	612993
Hu CD8 BUV615 SK1 100Tst	SK1	FCM	RUO	BUV615	100 Tests	612994
Hu CD8 BUV615 SK1 25Tst	SK1	FCM	RUO	BUV615	25 Tests	612995
Hu CD25 BUV615 2A3 100Tst	2A3	FCM	RUO	BUV615	100 Tests	612996
Hu CD25 BUV615 2A3 25Tst	2A3	FCM	RUO	BUV615	25 Tests	612997
Hu CD194 BUV615 1G1 100Tst	1G1	FCM	RUO	BUV615	100 Tests	613000
Hu CD56 BUV615 NCAM16.2 100Tst	NCAM16.2	FCM	RUO	BUV615	100 Tests	613001
Hu CD56 BUV615 NCAM16.2 25Tst	NCAM16.2	FCM	RUO	BUV615	25 Tests	613002

Anti-Mouse and Other Antibodies

Description	Clone	Apps	Reg	Format	Size	Cat No.
Ms CD8a BUV615 53-6.7 50ug	53-6.7	FCM	RUO	BUV615	50 µg	613004
Ms CD4 BUV615 GK1.5 50ug	GK1.5	FCM	RUO	BUV615	50 µg	613006
SAV BUV615 100ug	(none)	FCM	RUO	BUV615	0.1 mg	613013

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!