

Cell Proliferation and Apoptosis: Two Sides of a Coin

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Research Applications Support

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Cell Proliferation

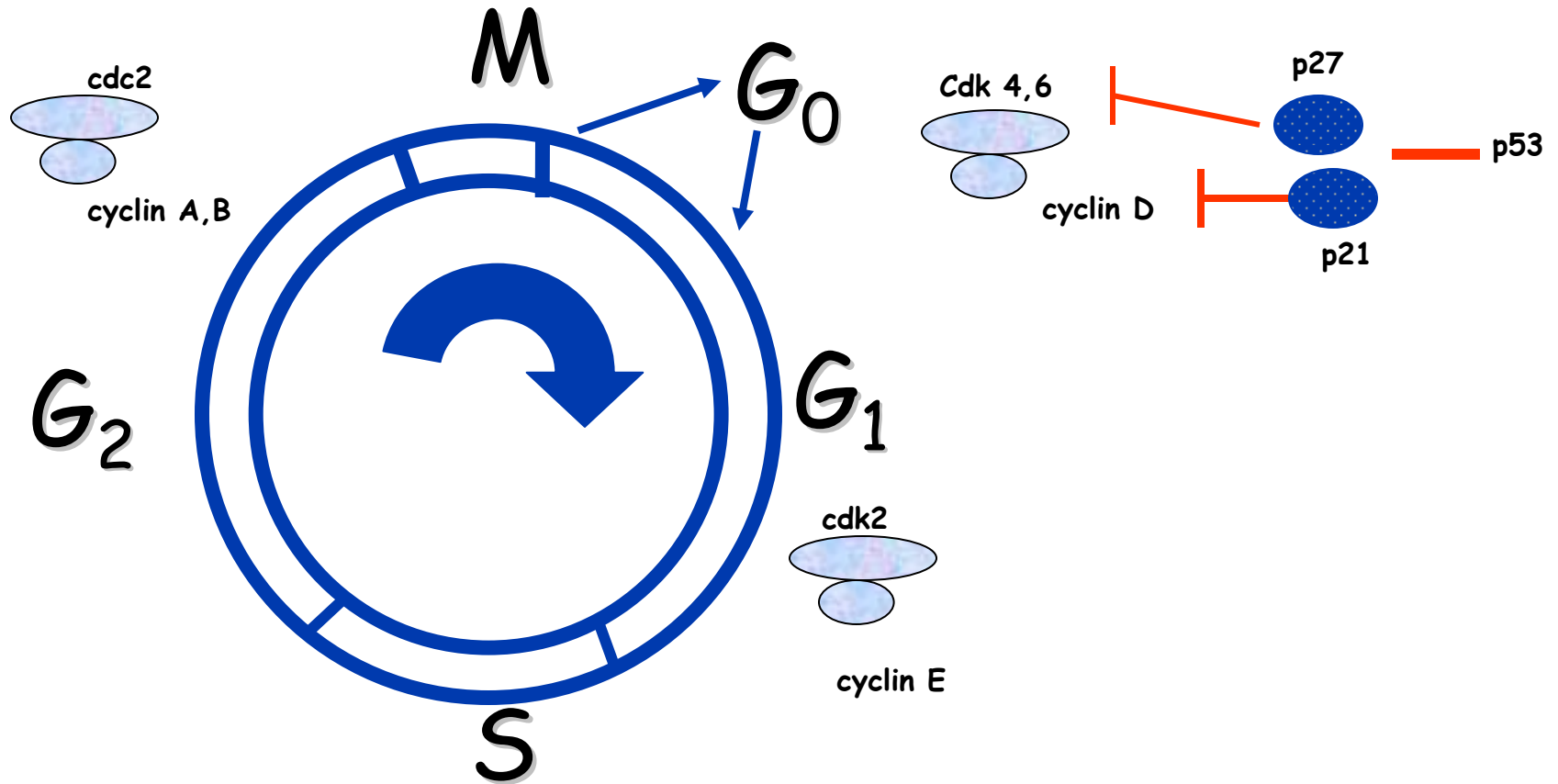
Cell proliferation is defined as an increase in the number of cells as a result of cell growth and division.

Uncontrolled cell growth or proliferation is the hallmark of cancer cells.

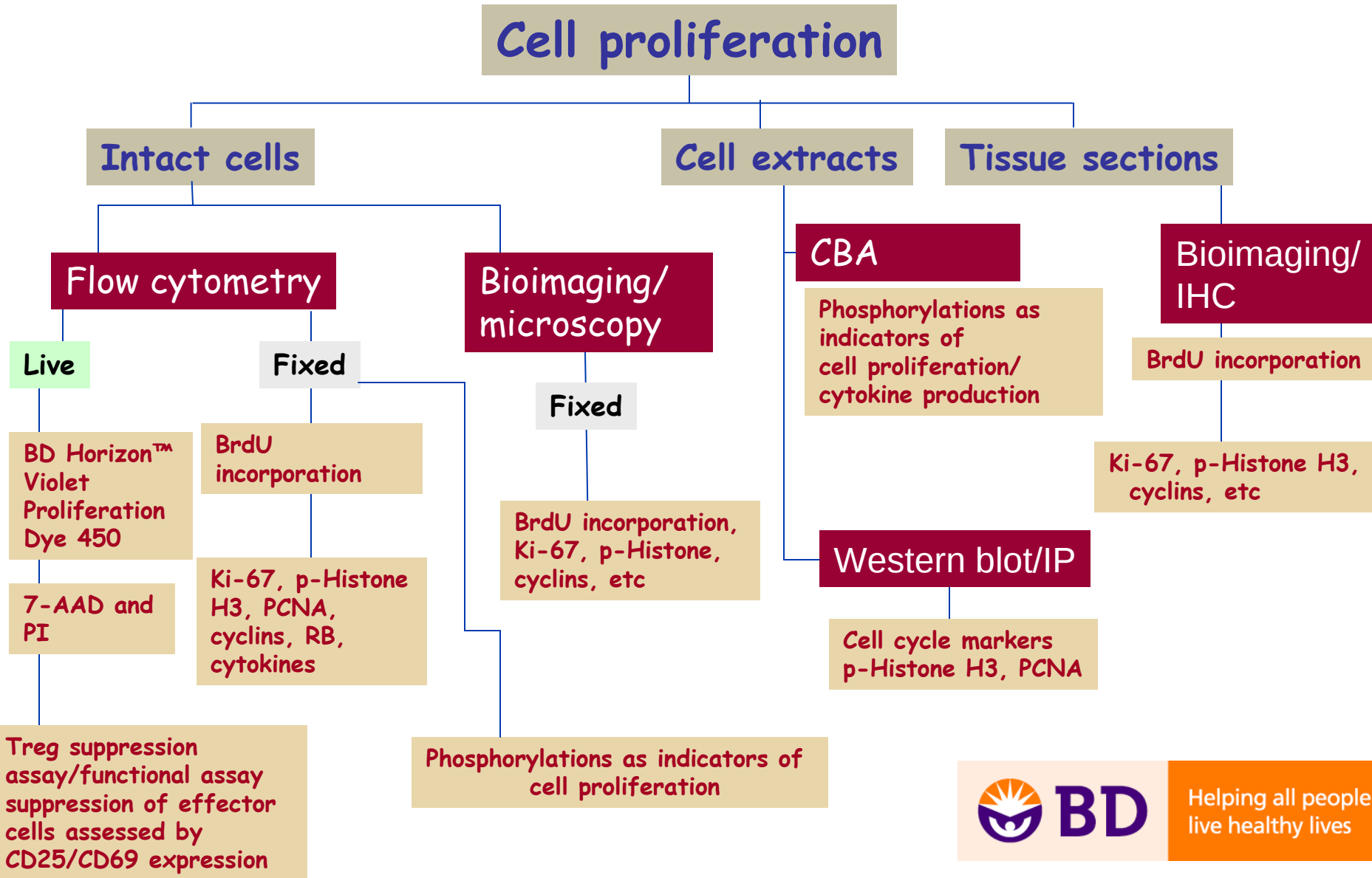


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The Cell Cycle and its Phases



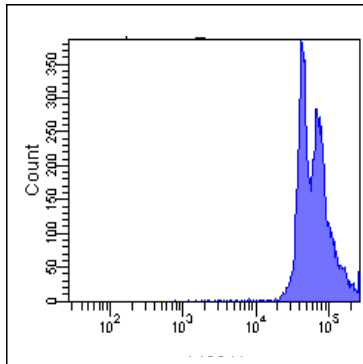
Cell Proliferation Application Decision Tree



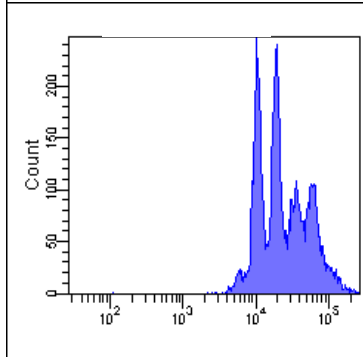
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Cell Proliferation Assessment Using Violet Proliferation Dye 450 (VPD 450)

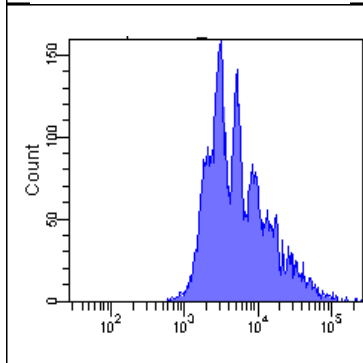
Day 1



Day 2



Day 3



VPD 450

Experimental design:

Enrich mouse spleen by positive selection via CD4⁺ enrichment.



Load isolated cells with VPD 450, 1 μ M, for 10 minutes.



Harvest CD3/CD28 stimulated cells on the days indicated.



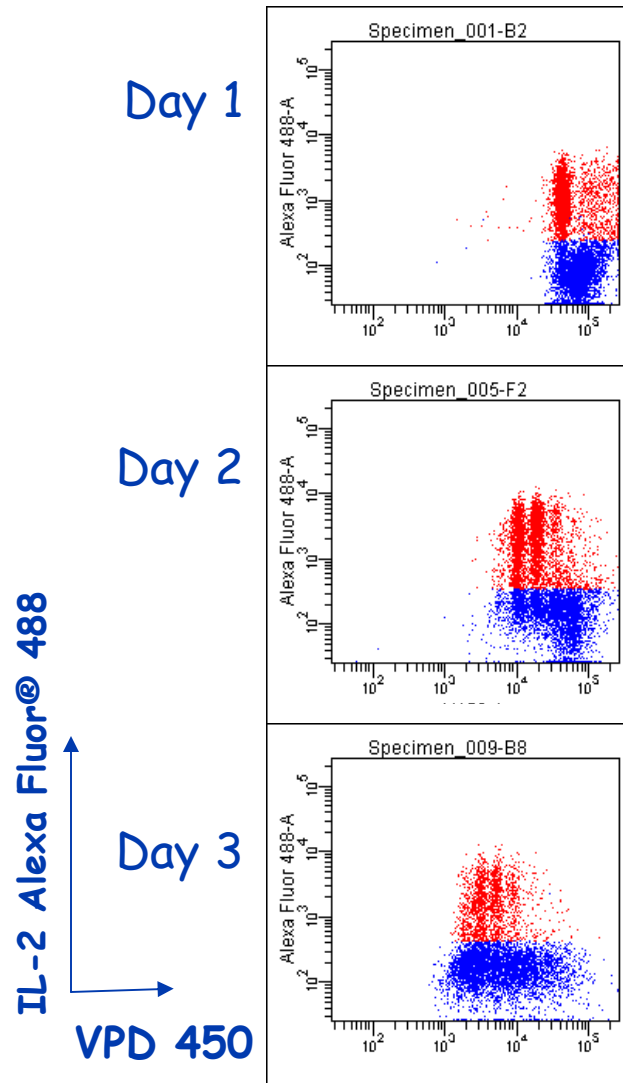
Analyze by flow cytometry.



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Simultaneous Assessment of Cell Proliferation and IL-2 Secreted by Cells During T-cell Stimulation

Condition: CD3/CD28



Experimental design:

Enrich Balb/c spleen by positive selection via CD4⁺ enrichment.



Load isolated cells with VPD 450, 1 μ M for 10 minutes.



Stimulate cells with soluble anti-CD3/CD28 (1 μ g) in the presence of transport inhibitor.

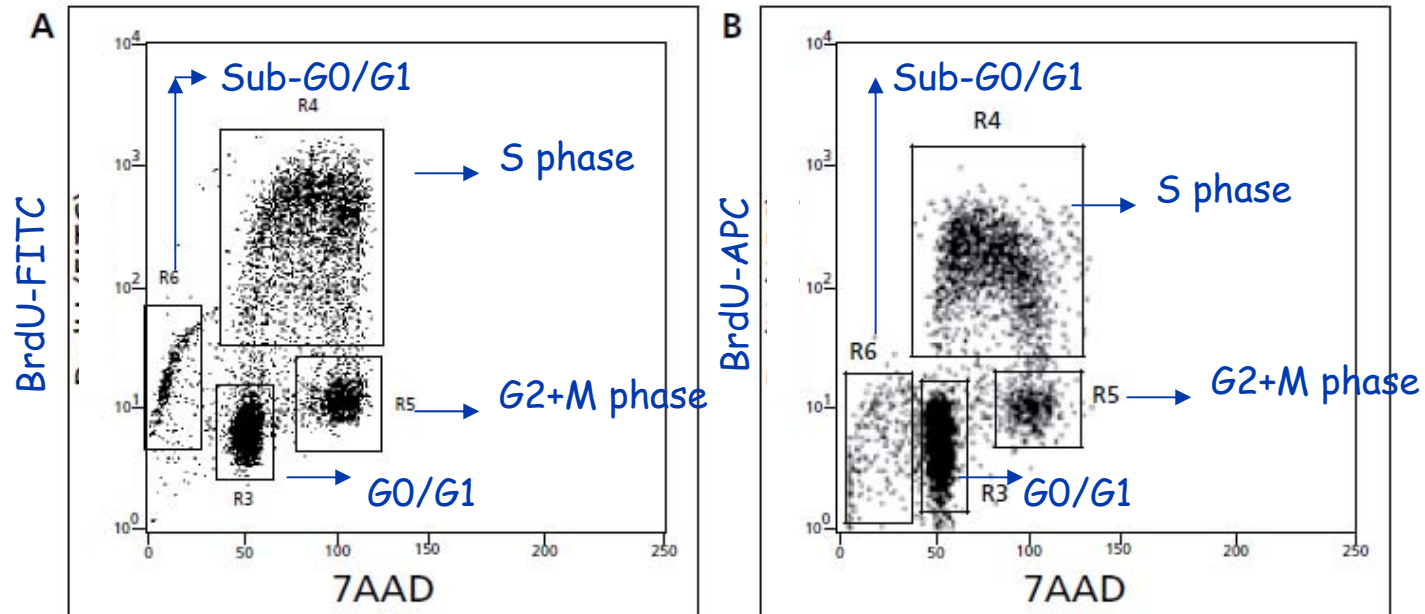


Fix/perm stain cells.



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Cell Cycle Analysis of Population Stained for Incorporated BrdU and Total DNA Levels (7-AAD)



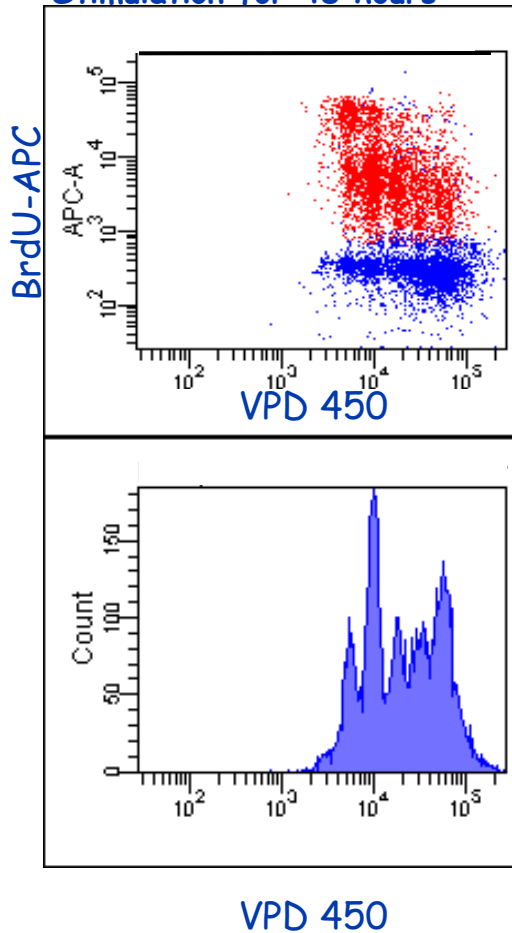
Human PBMCs were stimulated with anti-CD3/CD28 for 48 hours and re-stimulated with PMA+Ionomycin for 4 hours, and BrdU was added for the final 1 hour. Cells were then harvested and stained using the BrdU staining protocol.



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Stimulated Splenocytes Assessed for Cell Proliferation Using VPD 450 and anti-BrdU Ab Simultaneously

Condition: CD3/CD28
Stimulation for 48 hours



Experimental design:

Mouse splenocytes were incubated with 1 μ M VPD 450 for 10 minutes and stimulated with anti-CD3/CD28 for 48 hours. Cells were pulsed with BrdU for 1 hour, prior to harvest.

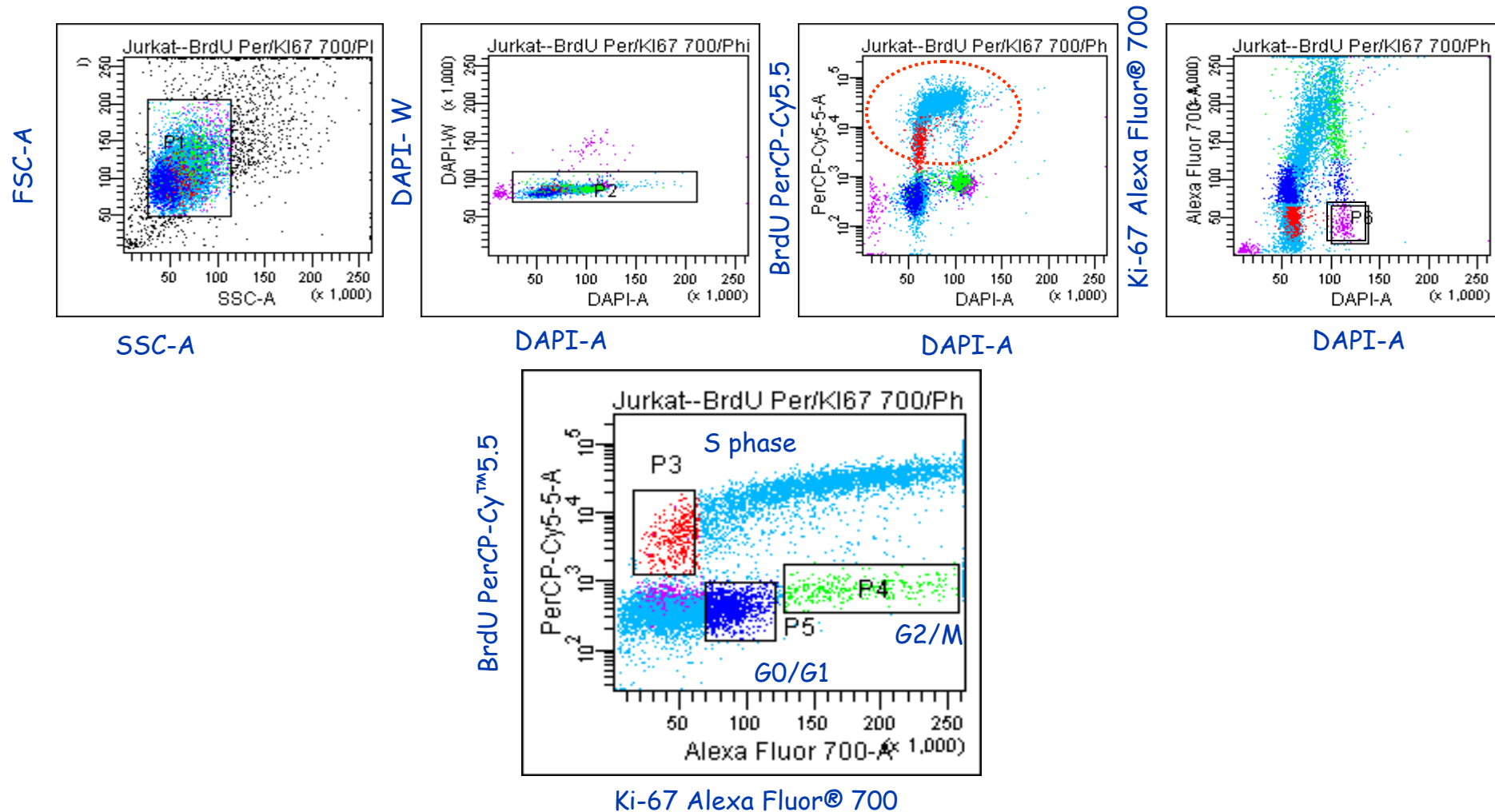


Cells were harvested and stained using the BrdU staining protocol and analyzed by flow cytometry.



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Ki-67: Another Marker for Cell Proliferation

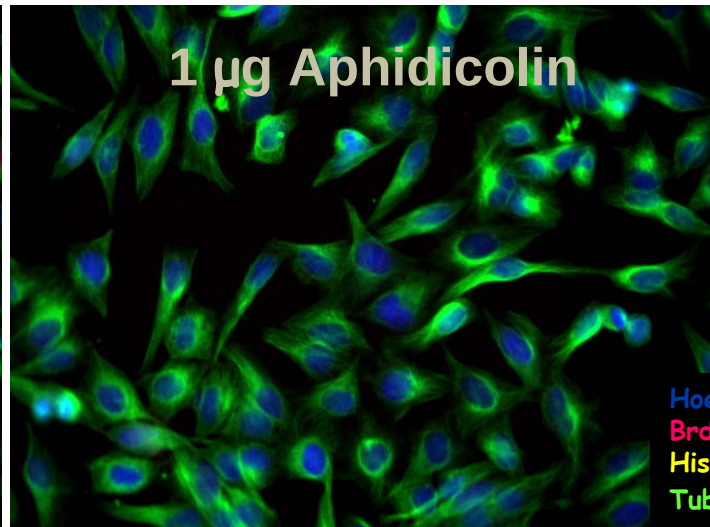
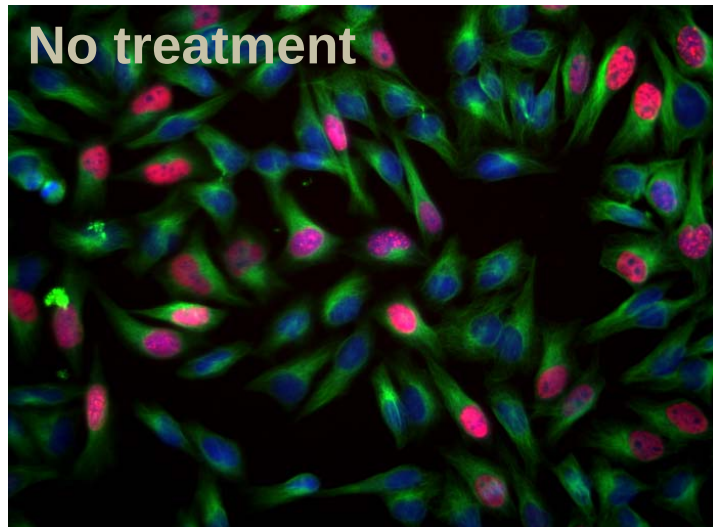


Ki-67 is expressed in G0/G1 (P5 gated cells) and post mitotic G2/M phase (P4 gated) cells (data generated at BD Biosciences).



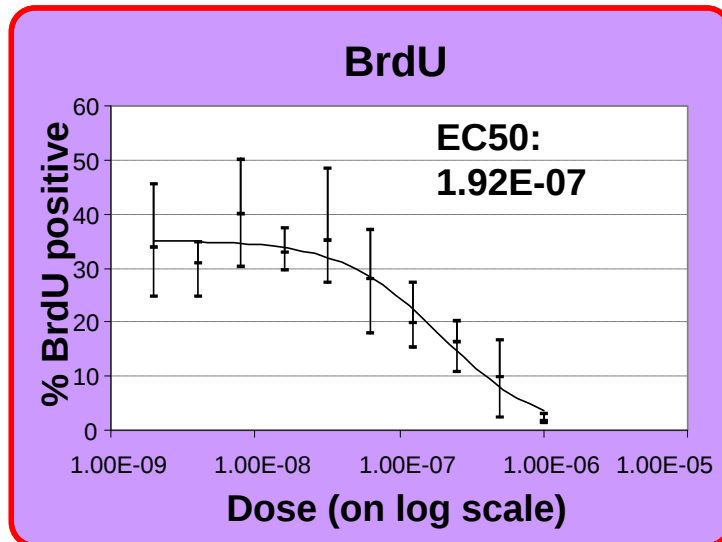
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Cell Cycle Analysis on HeLa Cells Treated with Aphidicolin (DNA Topoisomerase α Inhibitor) Monitored by BrdU Staining



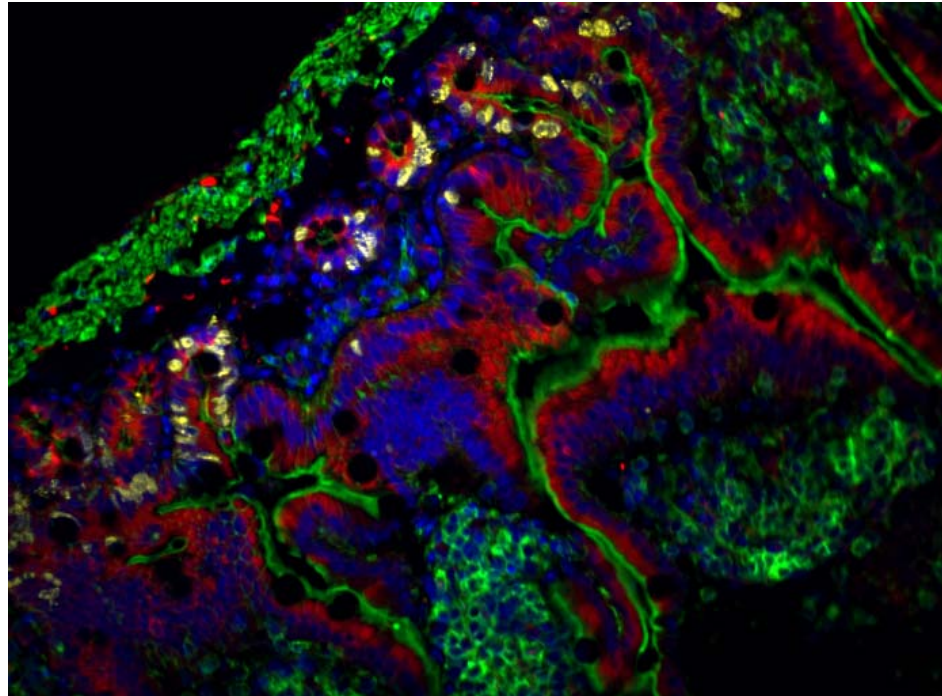
Hoechst	- blue
BrdU	- red
Histone	- yellow
Tubulin	- green

The images were captured on a BD Pathway™ 855 bioimaging system with a 20x objective and merged using BD Attovision™ software.



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Cell Proliferation Assessed in Mouse Small Intestinal Sections by BrdU Staining



Actin - green
 β -Tubulin- red
BrdU - yellow
Hoechst- blue

The images were captured on a BD Pathway™ 435 bioimaging system with a 20x objective and merged using BD Attovision software.



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Regulatory T Cells (Tregs)

Regulatory T cells, also called “Tregs,” play an important role in maintaining immunological **unresponsiveness** to self antigens (self tolerance) and control of immune responses to foreign antigens.

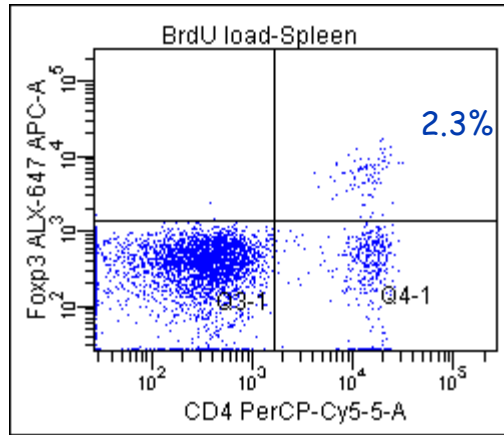
- Characteristics of Tregs: proliferate very slowly
- Hallmark assays to assess Treg functions
 - Suppress proliferation in effector cells
 - Suppress cytokine production by effector cells
- Salient markers for Tregs
 - Surface: CD4, CD25, CD127
 - Intracellular: FoxP3



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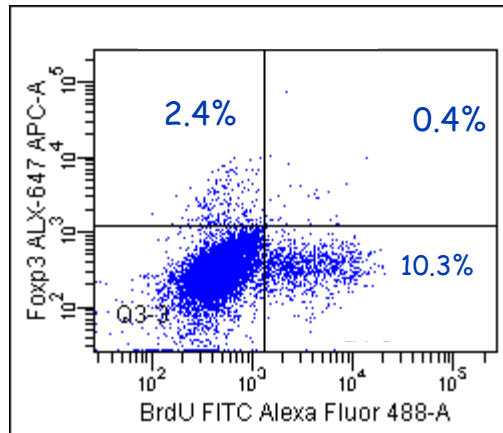
Staining of anti-BrdU with Foxp3 for Detection of Cell Proliferation in Mouse Tregs

Foxp3-Alexa Fluor® 647



CD4 PerCP-Cy5.5

Foxp3-Alexa Fluor® 647



BrdU FITC

Experimental design

Mouse splenocytes were stimulated with CD3/CD28 and cultured for 5 days. At the end of 5 days, cells were further cultured and then re-stimulated for 5 hours with PMA+Ionomycin. The cells were incubated with 100 μ M BrdU for the final hour of culture and then harvested.



Following harvesting, the cells were stained with anti-CD4 PerCP-Cy5.5 and Foxp3 Alexa Fluor® 647 using the Foxp3 staining protocol.



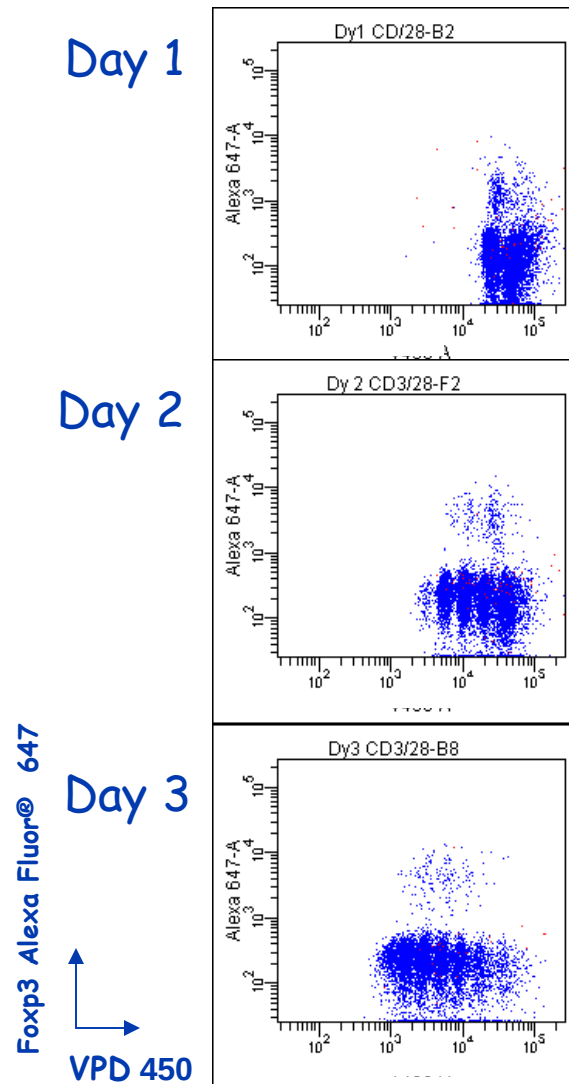
Upon completion of FoxP3 staining, the cells were refixed and permeabilized using BD Cytofix/Cytoperm™ buffer and stained with anti-BrdU Ab, allowing the detection of incorporated BrdU.



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Significant Insights into the Mechanism of Treg Proliferation as Assessed by Violet Proliferation Dye 450 (VPD 450)

Condition: CD3/CD28



Experimental design

Enrich mouse splenocytes by positive selection via CD4⁺ enrichment.



Load isolated cells with VPD 450, 1 μ M, for 10 minutes. CD3/CD28 stimulated cells were harvested on days as indicated



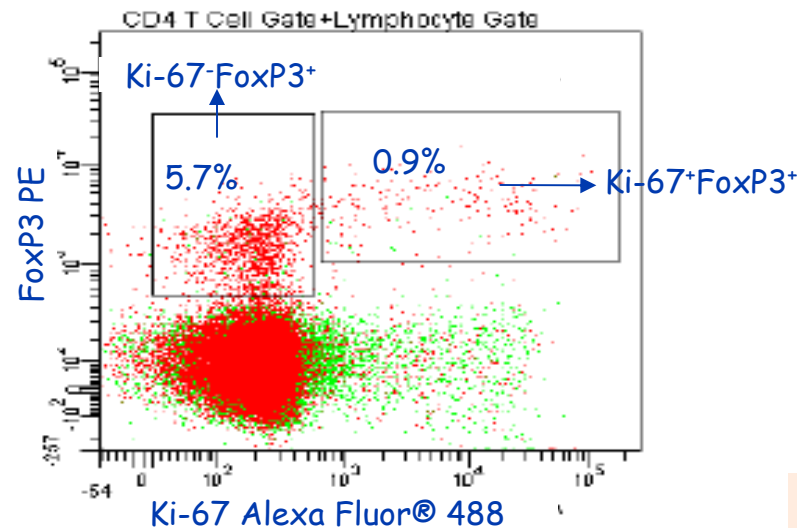
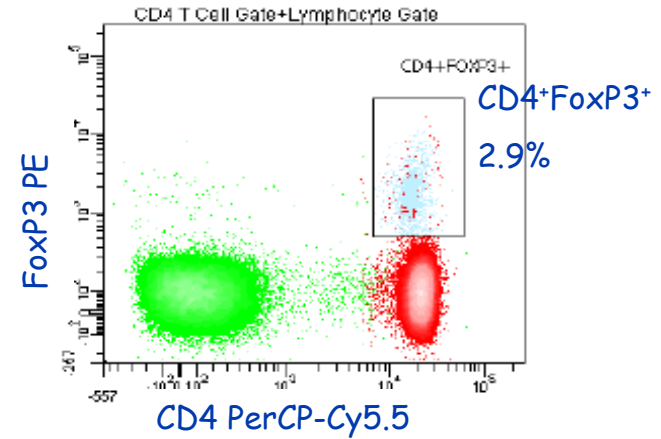
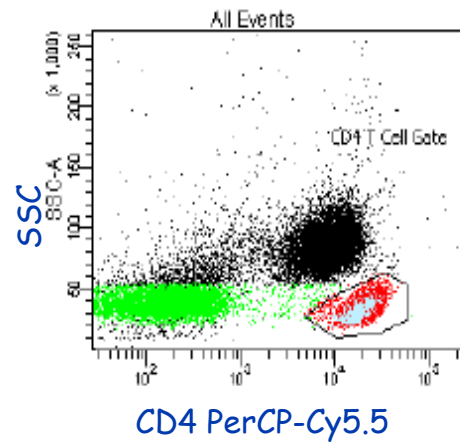
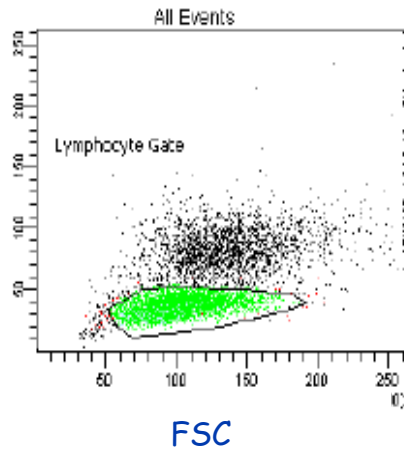
Fix and permeabilize cells using the Foxp3 staining protocol.



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Expression of Ki-67 in Human Tregs

SSC



Human PBMCs were stained for Ki-67 and FoxP3 using the FoxP3 staining protocol.



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New Assay to Assess Treg Function: Suppression of Effector Cells

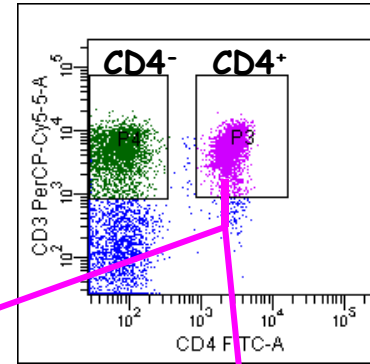
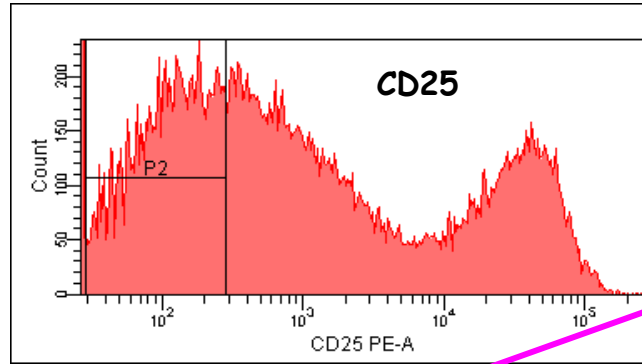
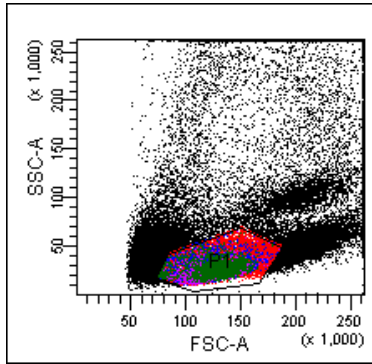
Treg Suppression Assay Kit: how the assay works

- Tregs are sorted using CD4⁺, CD25⁺, CD127^{low/dim} and CD45RA⁺.
- Cells are expanded in culture for 13 days.
- Expanded Tregs are placed with effector cells (autologous PBMCs) in the presence of T-cell specific stimulus (SEB, CD3/CD28, CD2/CD2R).
- After 7 hours, the frequency of CD69-positive and/or CD154-positive effector T cells (response) is measured in the presence and absence of Tregs.
- CD25 is used to identify and exclude Tregs during analysis.
- The percent suppression of the response is calculated.



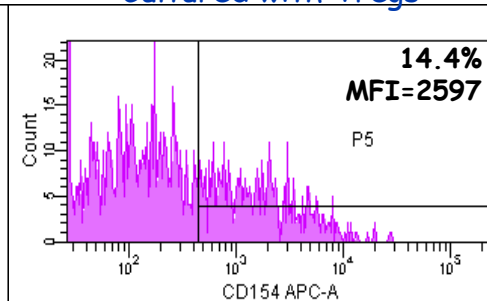
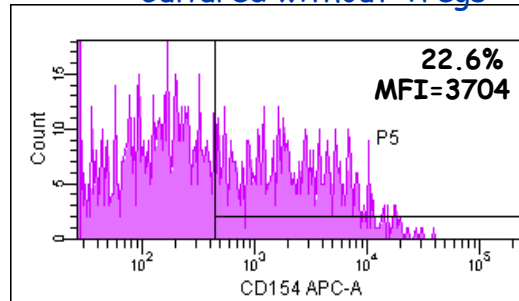
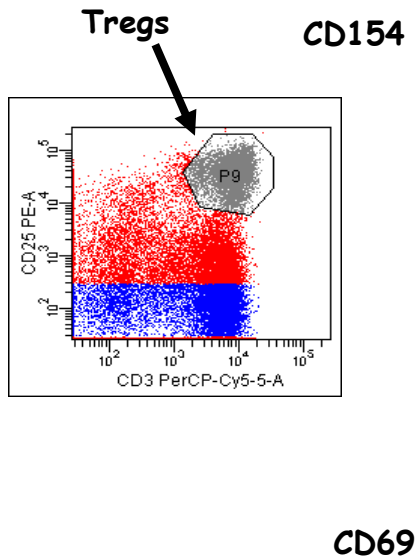
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Treg-mediated Suppression of CD154 and CD69 in PBMCs Stimulated with CD3/CD28

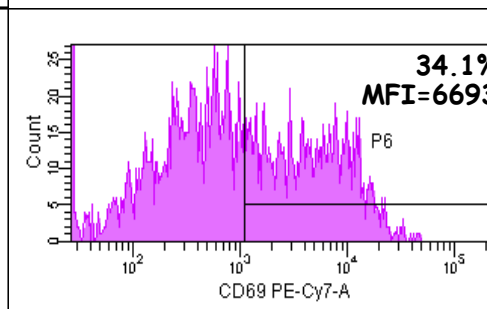
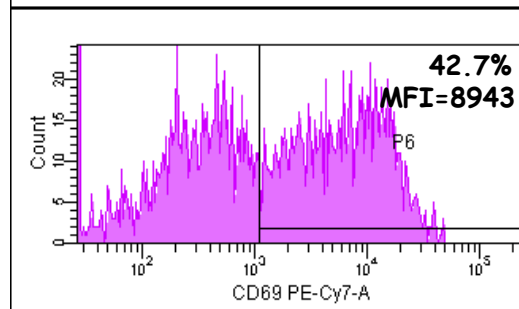


Cultured without Tregs

Cultured with Tregs



CD154



CD69



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Apoptosis

Definition: The process leading to controlled self-destruction of a cell. Cells undergo death neatly without damaging their neighbors. Apoptosis is a "programmed event."



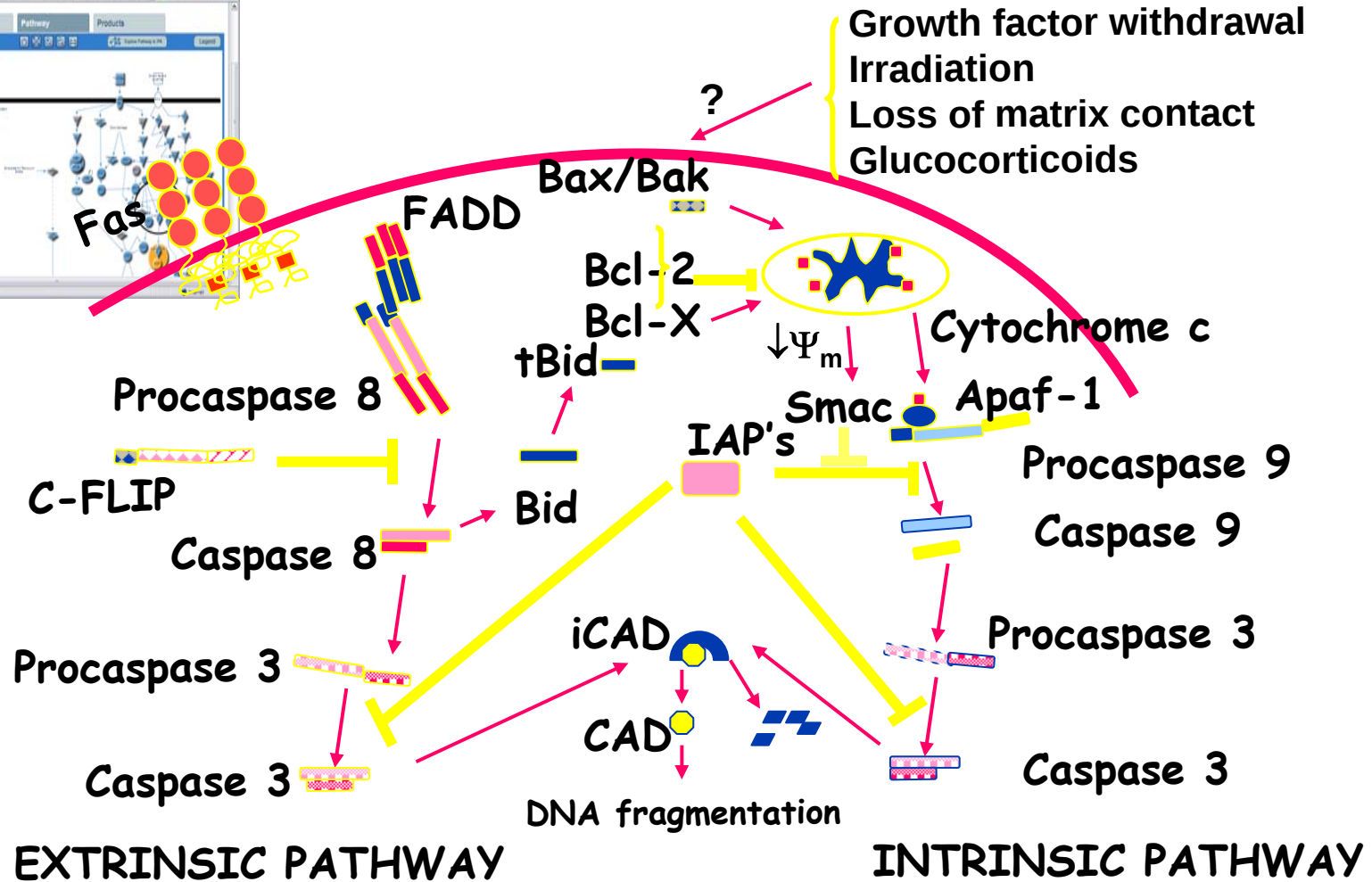
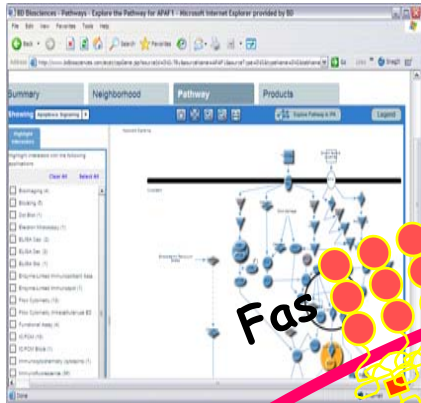
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Importance of Apoptosis

- **Development**
 - Organs, appendages, patterning
 - Thymic selection (lymphocyte development)
- **Tissue homeostasis**
 - Tumor
- **Cell termination**
 - Viral infection, cancer

Apoptotic Signaling Pathways

bdbiosciences.com/pathways



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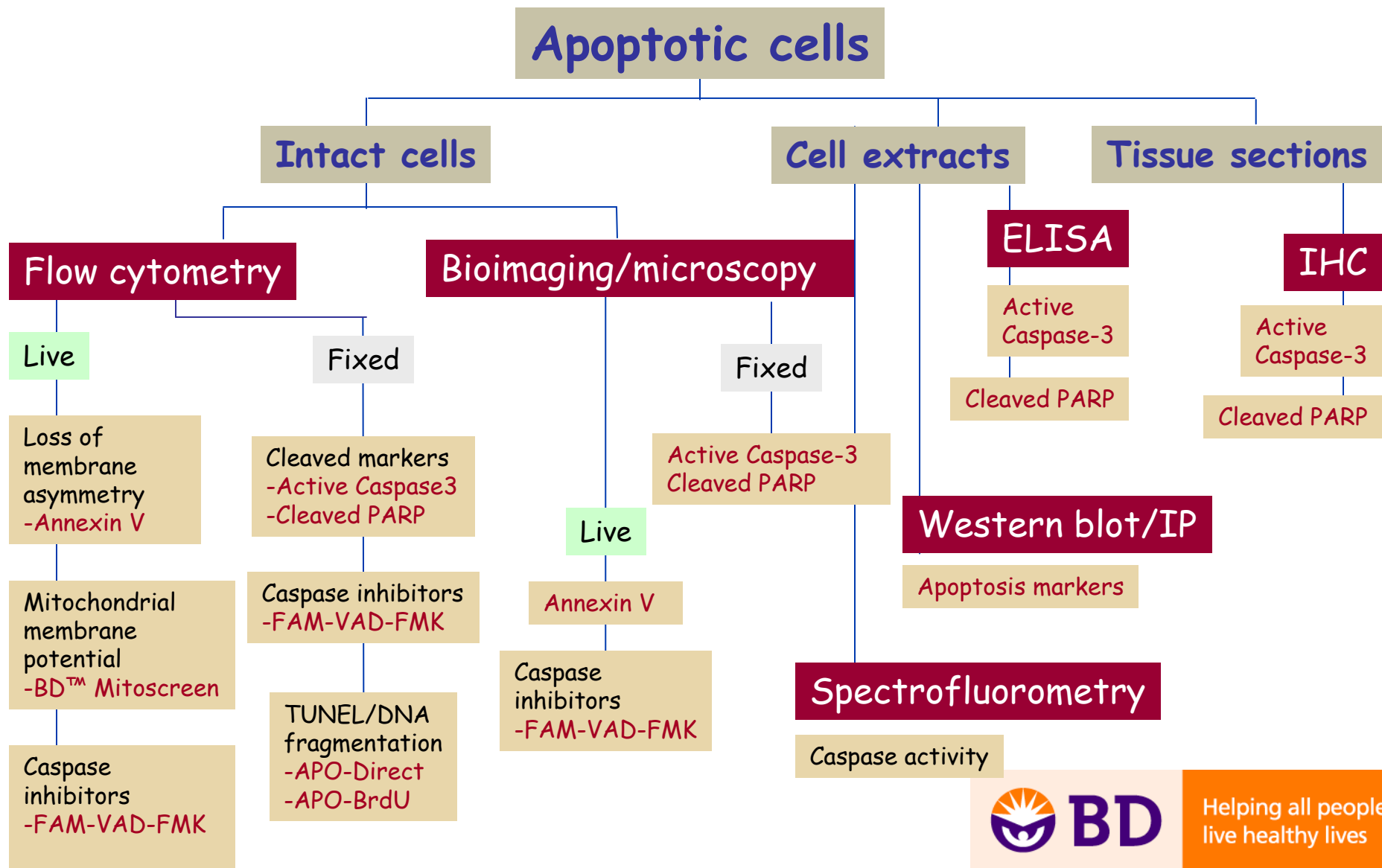
Hallmarks of Apoptosis

- Plasma membrane alterations
- Mitochondrial changes
- Activation of caspases
- DNA fragmentation



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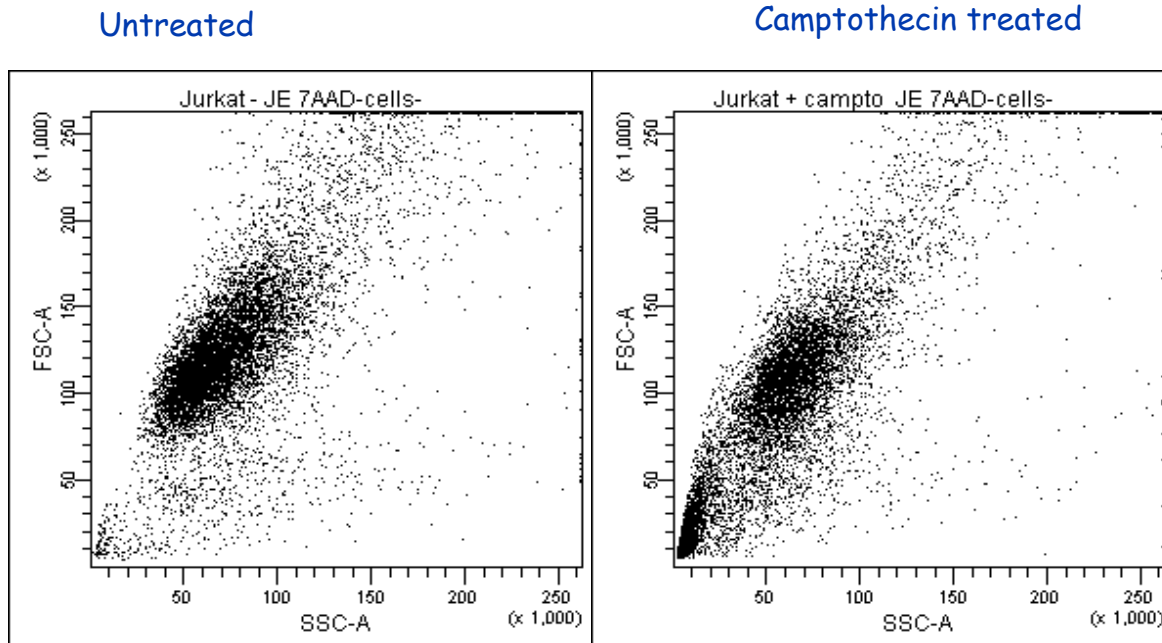
Apoptosis Application Decision Tree



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Apoptosis: Scatter Properties

Cell shrinkage during apoptosis is associated with a decrease in forward scatter. Analysis of light scatter is often combined with other assays.



Formation of apoptotic vesicles

- **Increases side scatter**

Reduced refractive index of apoptotic cells

- **Decreases forward scatter**



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Annexin V

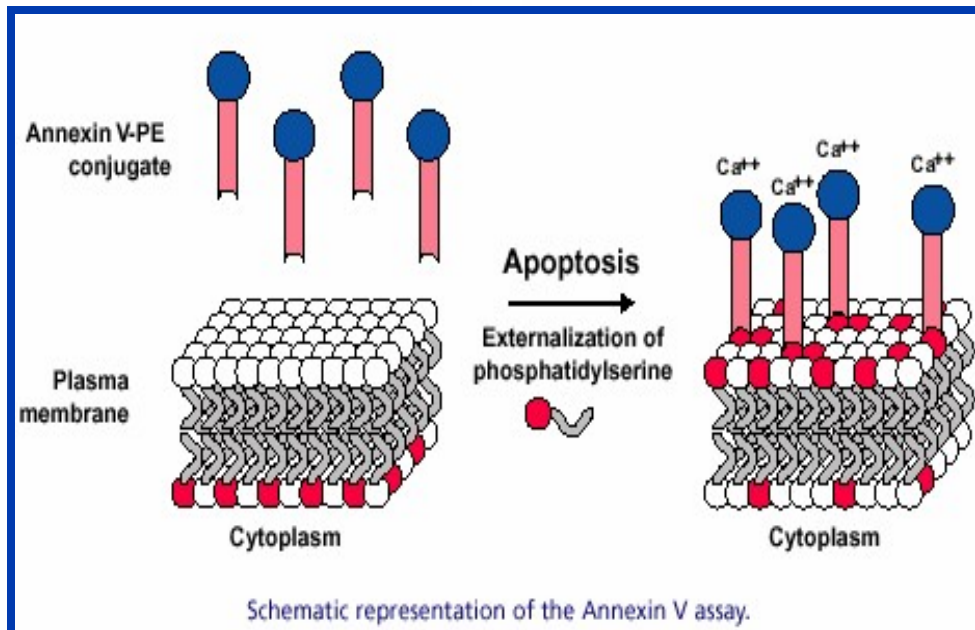
Annexin V is a surface marker and detects early membrane changes associated with apoptosis.

Pros: Rapid confirmation of apoptosis

Uses live, unfixed cells

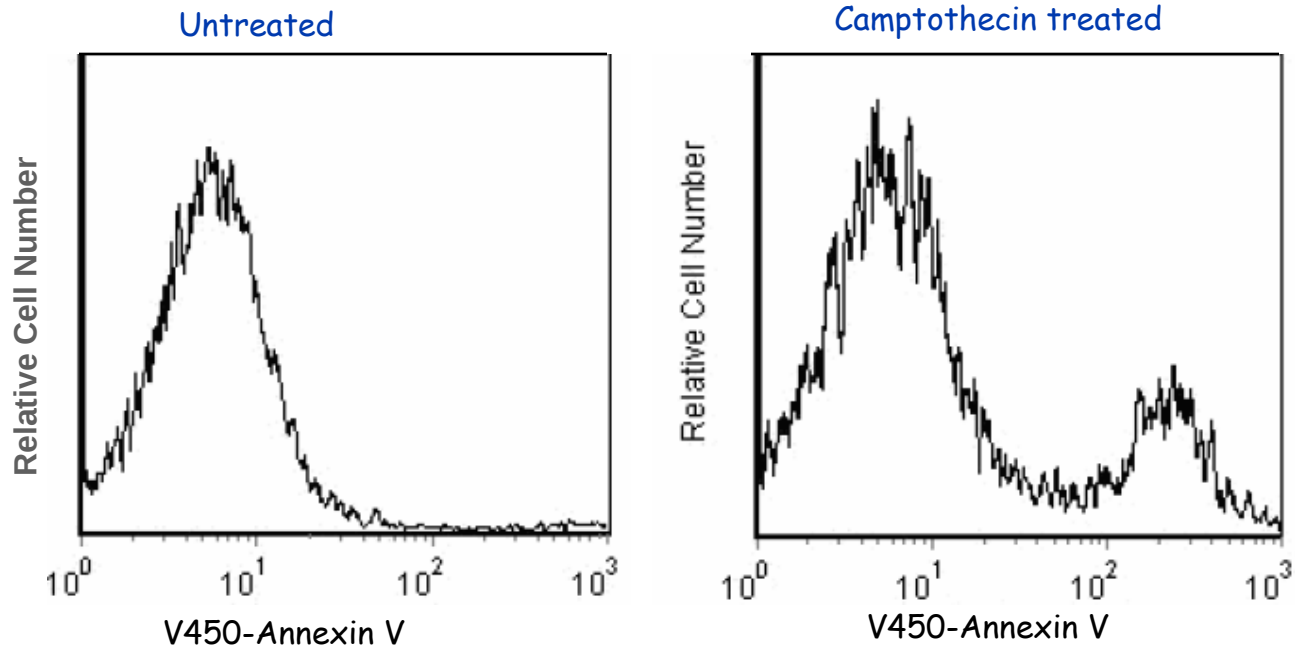
Applications

- Flow cytometry (cells in suspension)
- Fluorescence microscopy (adherent cells)



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Detection of Membrane Changes by Annexin V Staining and Analysis by Flow Cytometry



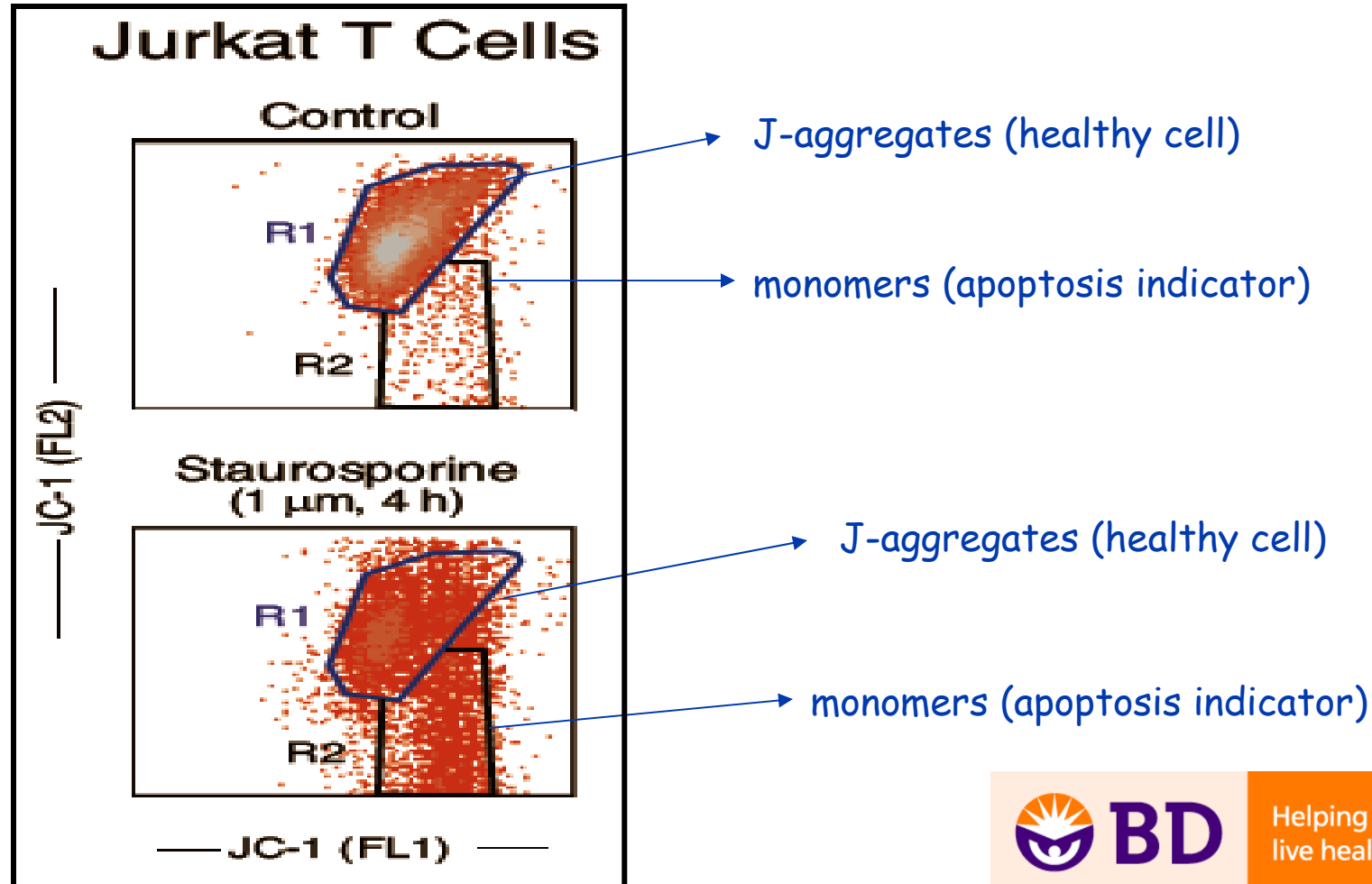
Jurkat T cells were treated with 6 μ M camptothecin for 4 hours. Cells were incubated with BD Horizon™ V450 Annexin V and analyzed by flow cytometry.



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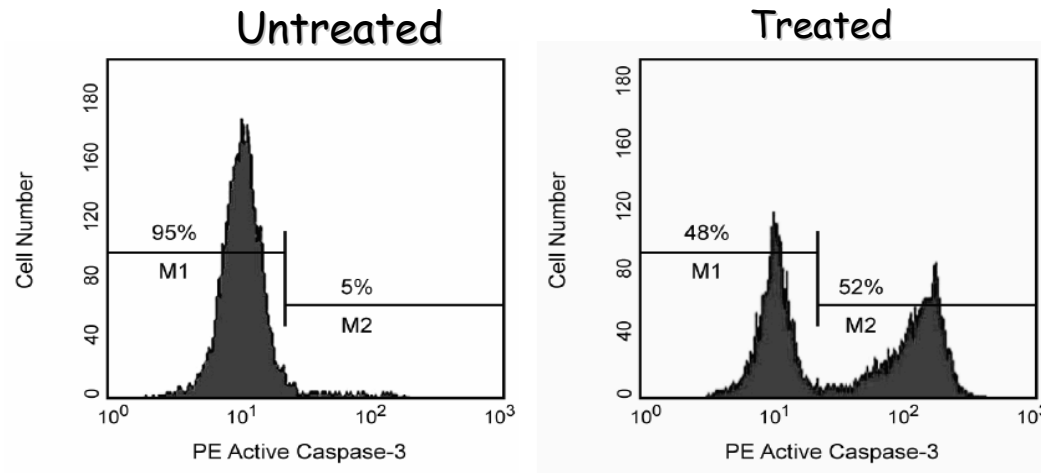
Detection of Changes in Mitochondrial Membrane Potential (JC-1)

JC-1: lipophilic cationic dye fluorescence is detected on a flow cytometer

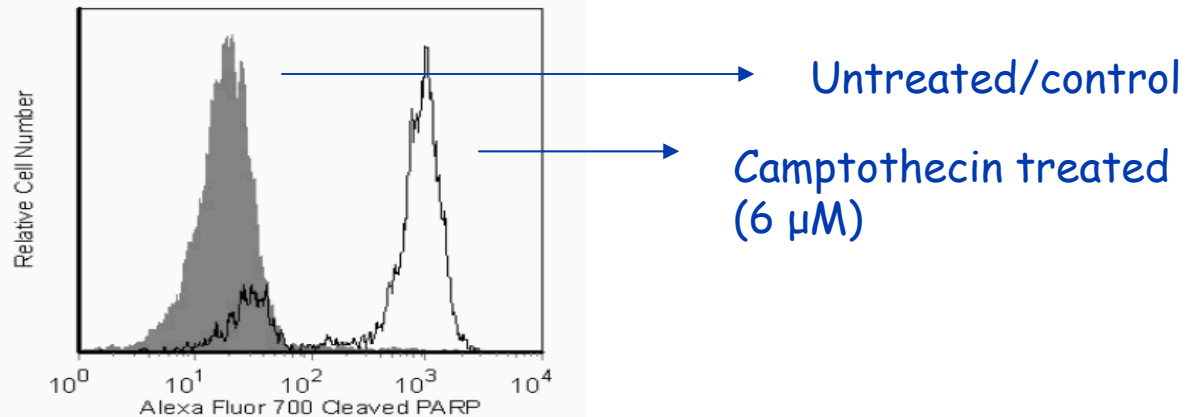


Detection of Active Caspase 3: "Executioner" of Apoptosis and its By-product: Cleaved PARP

Caspase-3



Cleaved-PARP

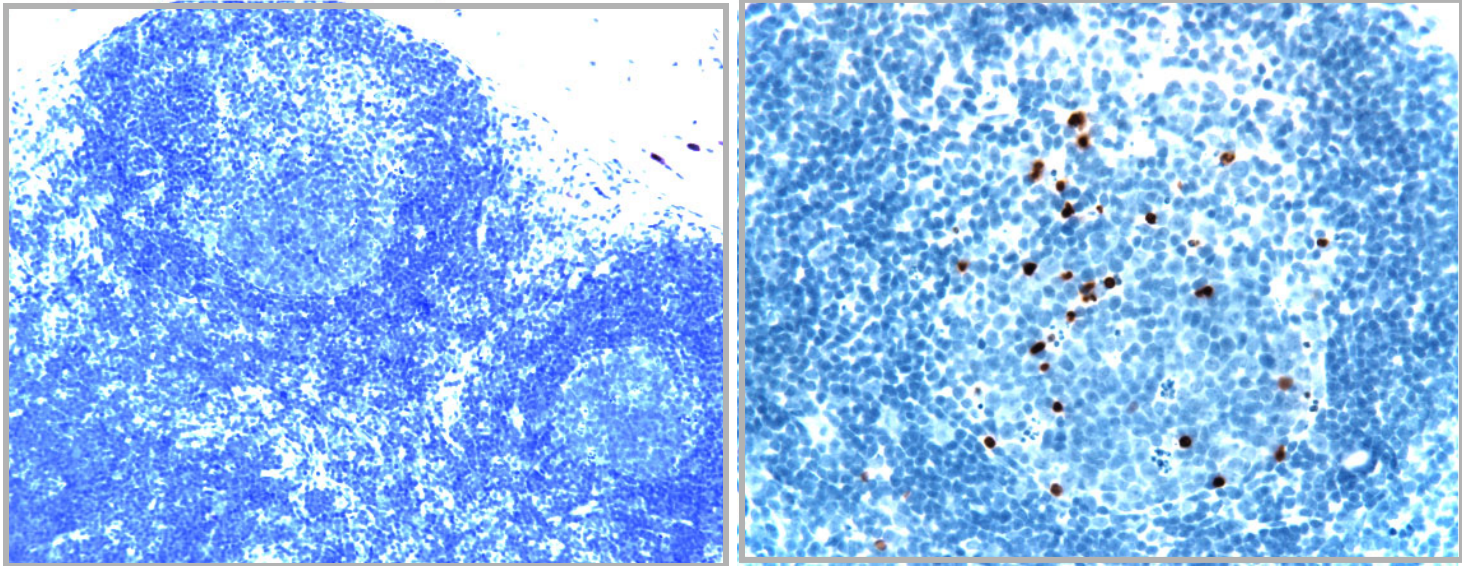


Jurkat T cells were treated with camptothecin, fixed and permeabilized with BD Cytotfix/Cytoperm buffer, and subsequently stained for active caspase 3 using anti-caspase Ab or cleaved PARP.



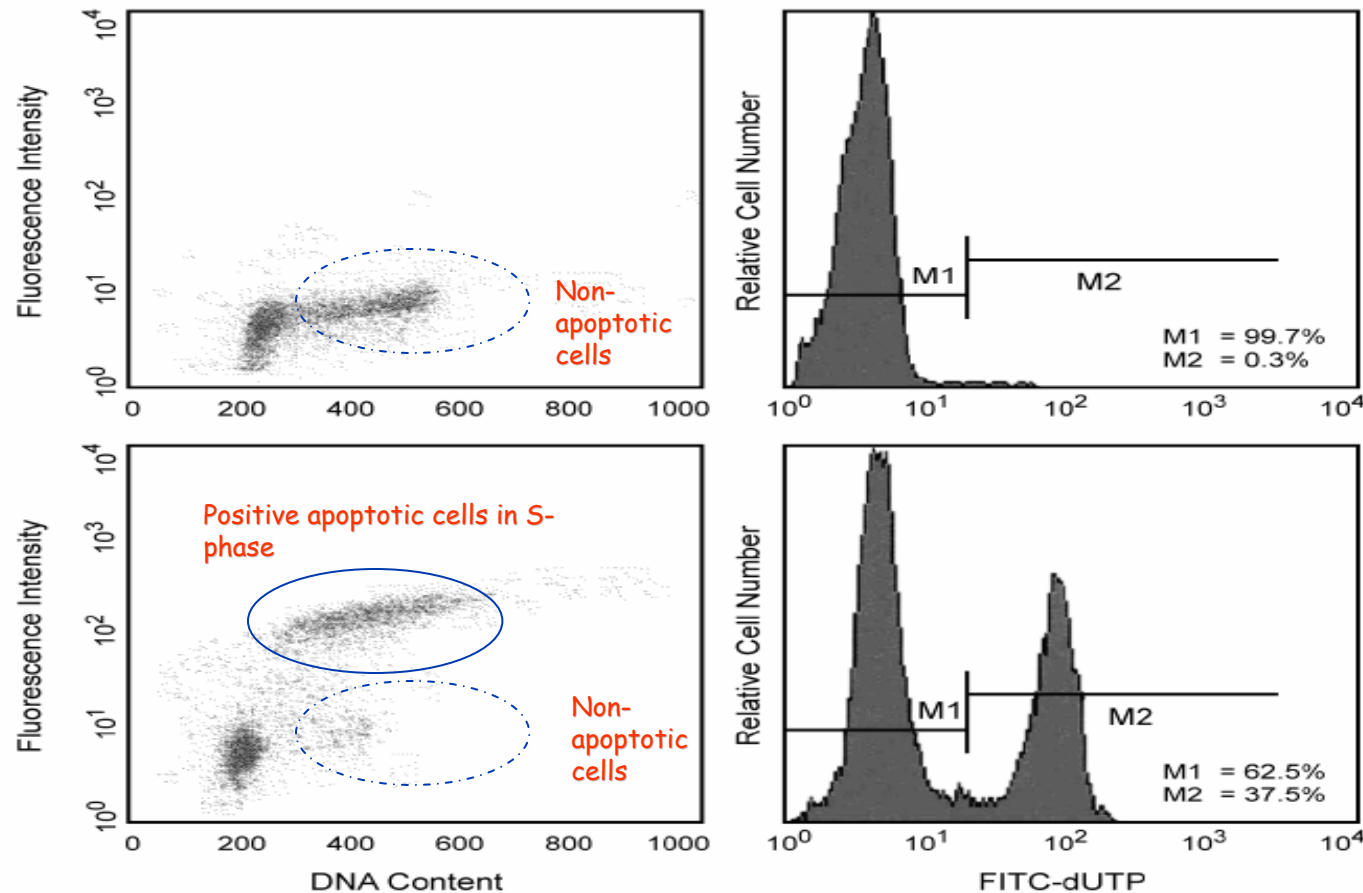
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Cleaved PARP Expression in Formalin-fixed, Paraffin-embedded Rat Lymph Node



Rat lymph nodes were stained with monoclonal cleaved PARP specific Ab F21-852 using the biotin, streptavidin three-step detection method.

Detection of DNA Fragmentation During Apoptosis by "End Labeling" or "TUNEL" Using the APO-DIRECT™ Kit



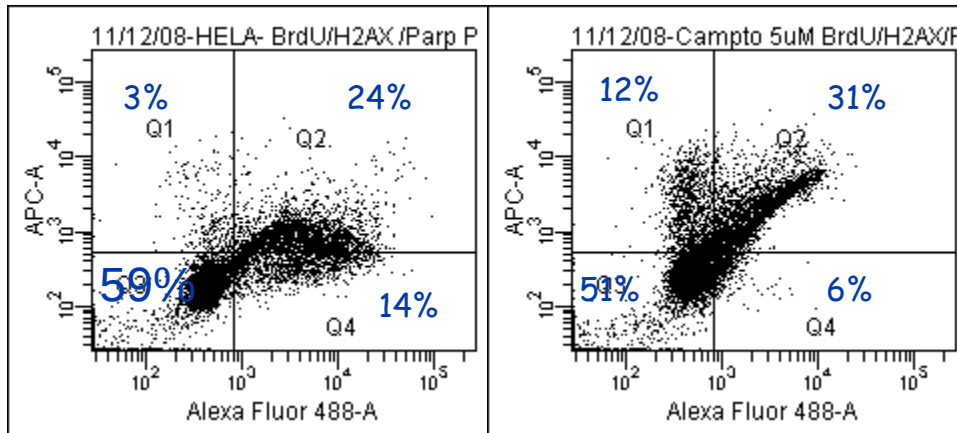
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Significant Tool in Drug Discovery Research: Assessing Cell Proliferation, DNA Damage, and Apoptosis Using Flow Cytometry

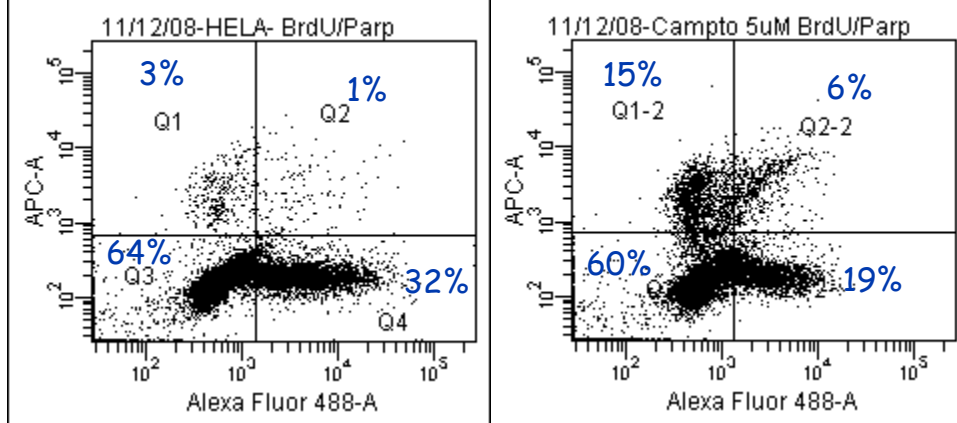
Untreated

Camptothecin treatment, 5 μ M

Phos H2AX-APC



Cleaved PARP-APC



BrdU Alexa Fluor® 488

Experimental design

HeLa cells were untreated or treated with camptothecin 5 μ M and BrdU for 4 hours.



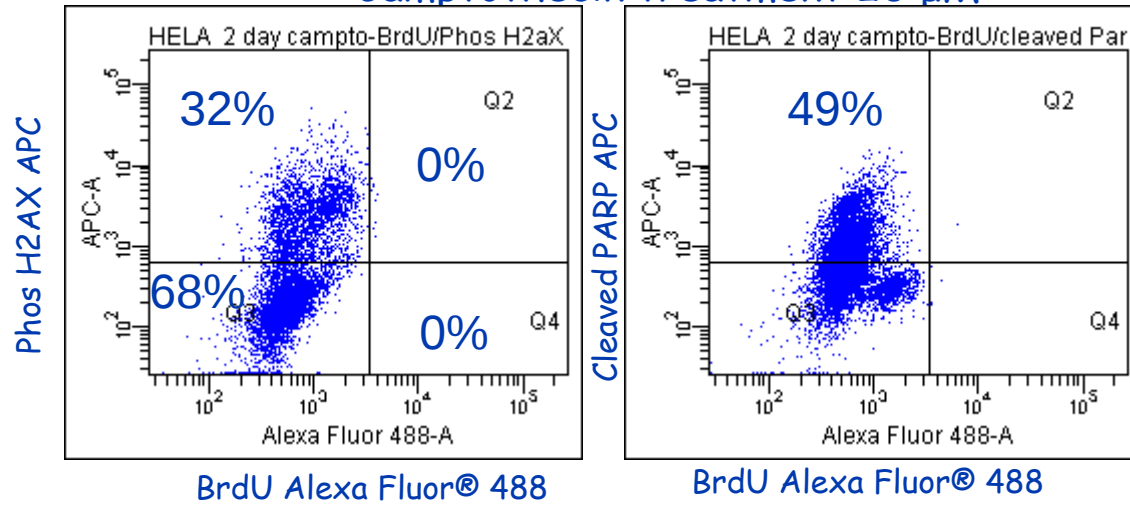
Cells were then harvested and stained with anti-BrdU, H2AX, and cleaved PARP using the BrdU staining protocol.



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Increase in Camptothecin Concentration and Incubation Time Leads to Increased H2AX and PARP Expression and Loss of BrdU Incorporation

Camptothecin treatment 20 μ M



Experimental design

HeLa cells were untreated or treated with camptothecin, 20 μ M, for 24 hours, further incubated for 48 hours post washing, and pulsed with BrdU for the final 1 hour.



Cells were then harvested and stained with anti-BrdU, H2AX, and cleaved PARP using the BrdU staining protocol.



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Acknowledgments

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