Cell Proliferation and Apoptosis: Two Sides of a Coin

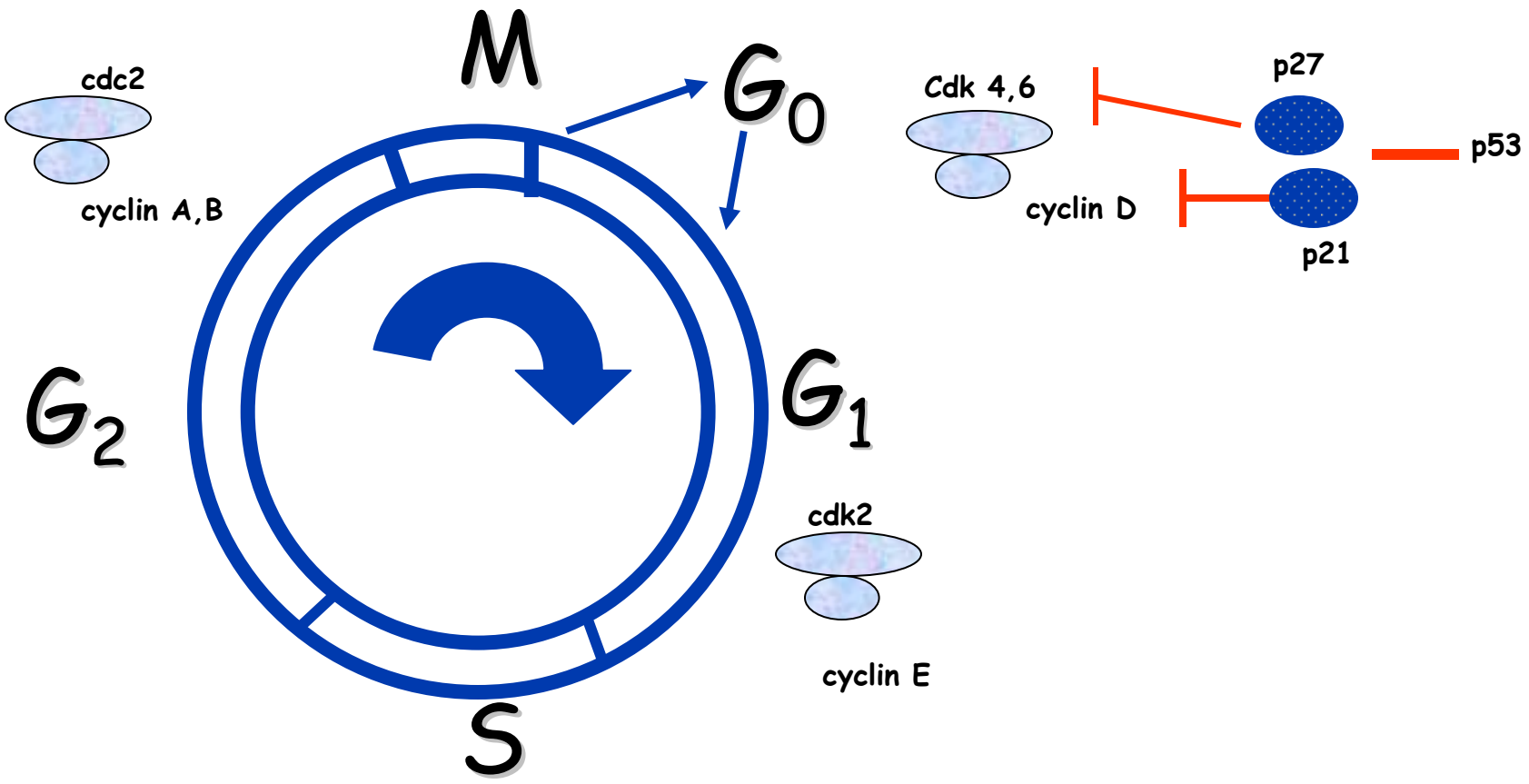
Monisha Sundarrajan, PhD
Senior Scientist
Research Applications Support

Cell Proliferation

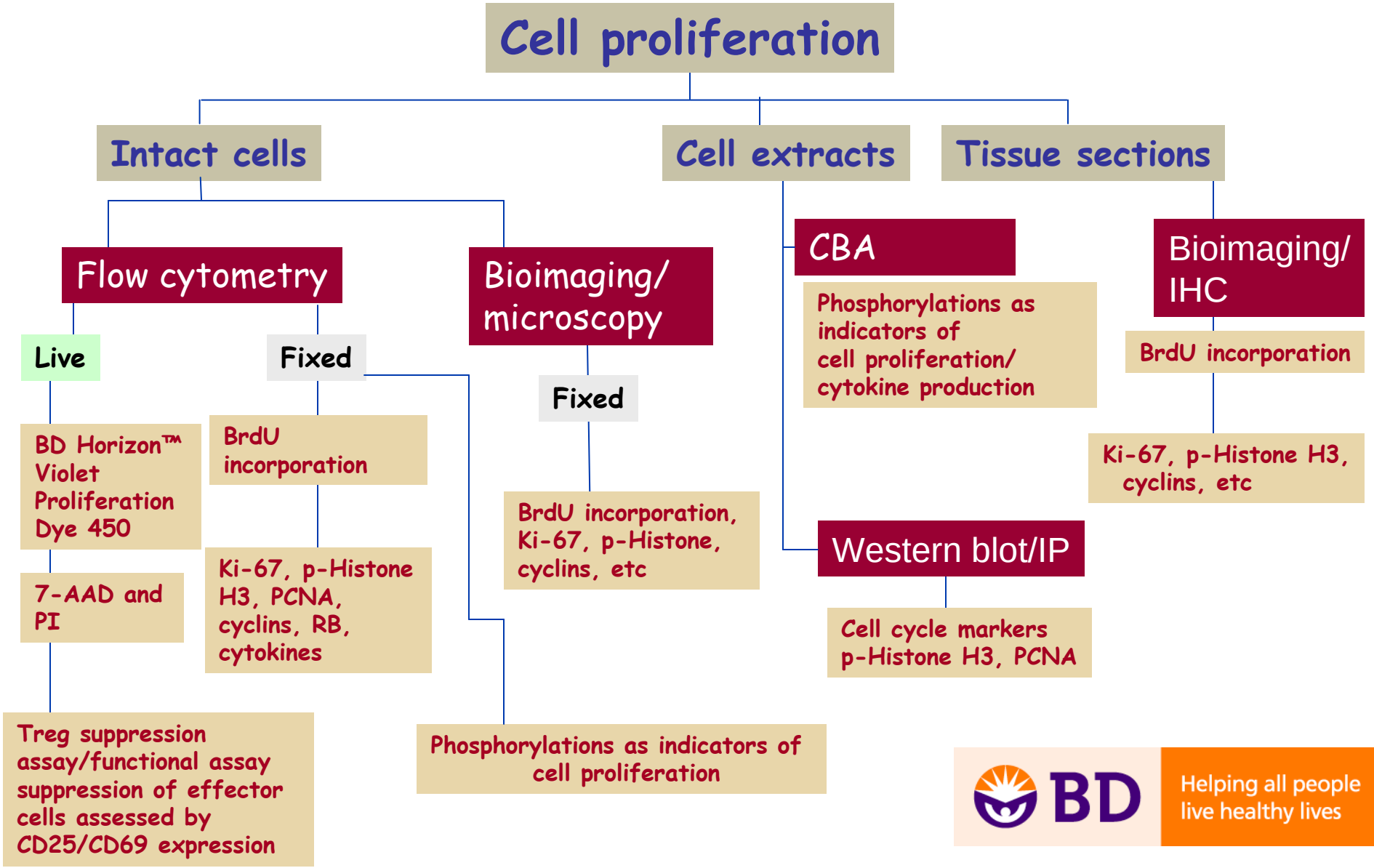
Cell proliferation is defined as an increase in the number of cells as a result of cell growth and division.

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

The Cell Cycle and its Phases



Cell Proliferation Application Decision Tree



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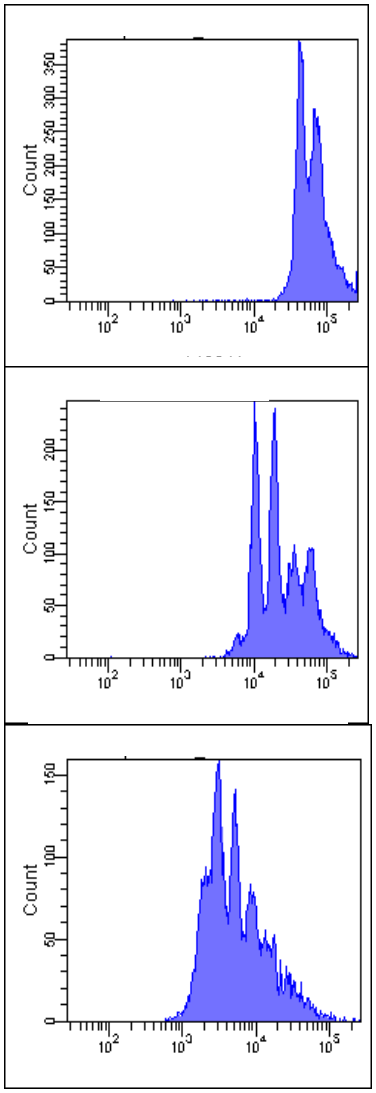
Cell Proliferation Assessment Using Violet Proliferation Dye 450 (VPD 450)

Day 1

Day 2

Day 3



Experimental design:

Enrich mouse spleen by positive selection via $CD4^+$ enrichment.

↓

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

↓

Harvest CD3/CD28 stimulated cells on the days indicated.

↓

Analyze by flow cytometry.

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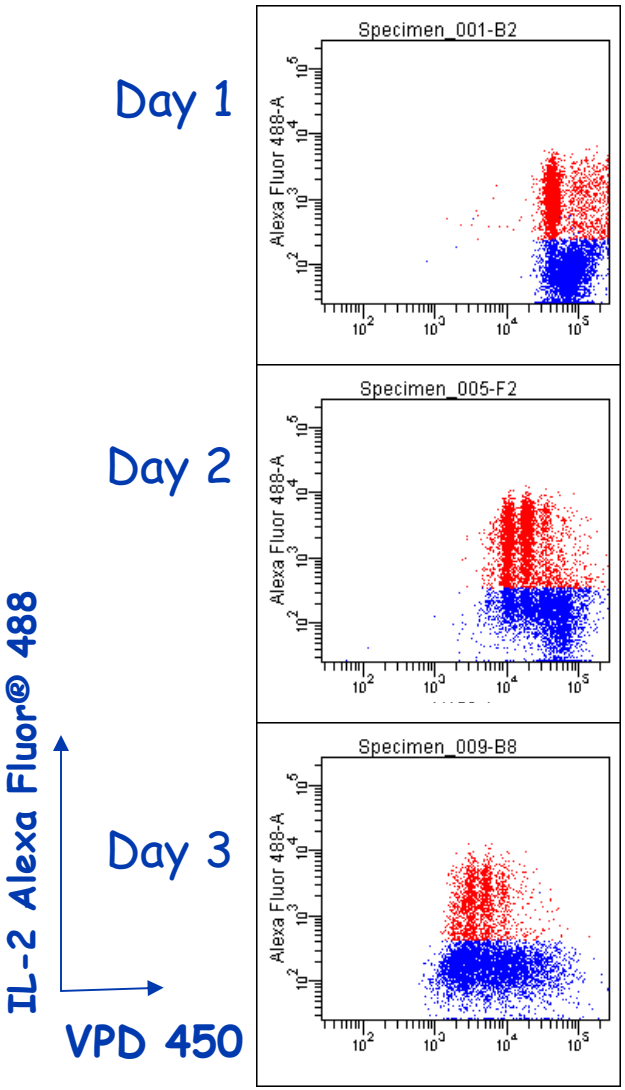
Simultaneous Assessment of Cell Proliferation and IL-2 Secreted by Cells During T-cell Stimulation

Condition: CD3/CD28



Experimental design:

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

↓

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

↓

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

↓

Fix/perm stain cells.

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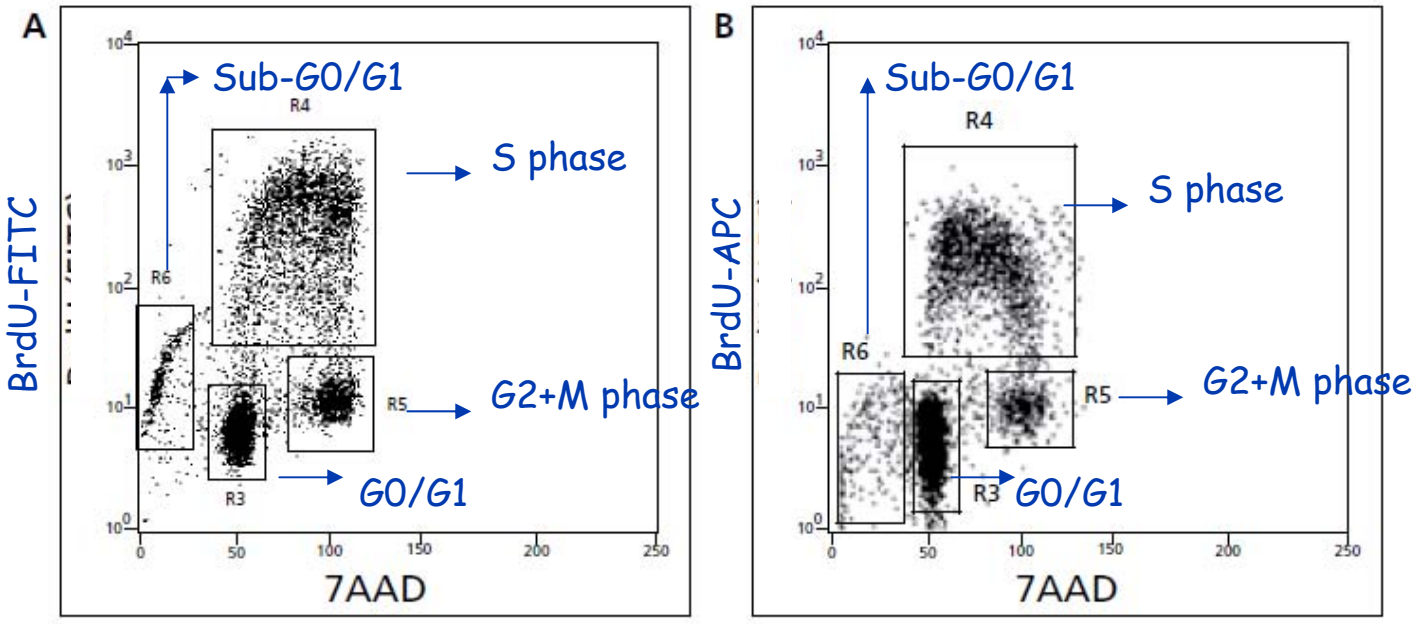
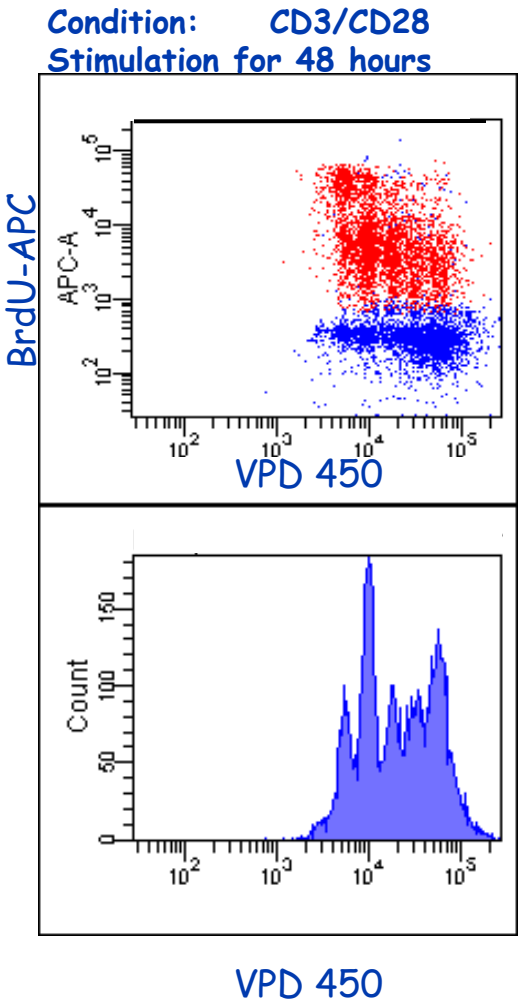
Cell Cycle Analysis of Population Stained for Incorporated BrdU and Total DNA Levels (7-AAD)



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.

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

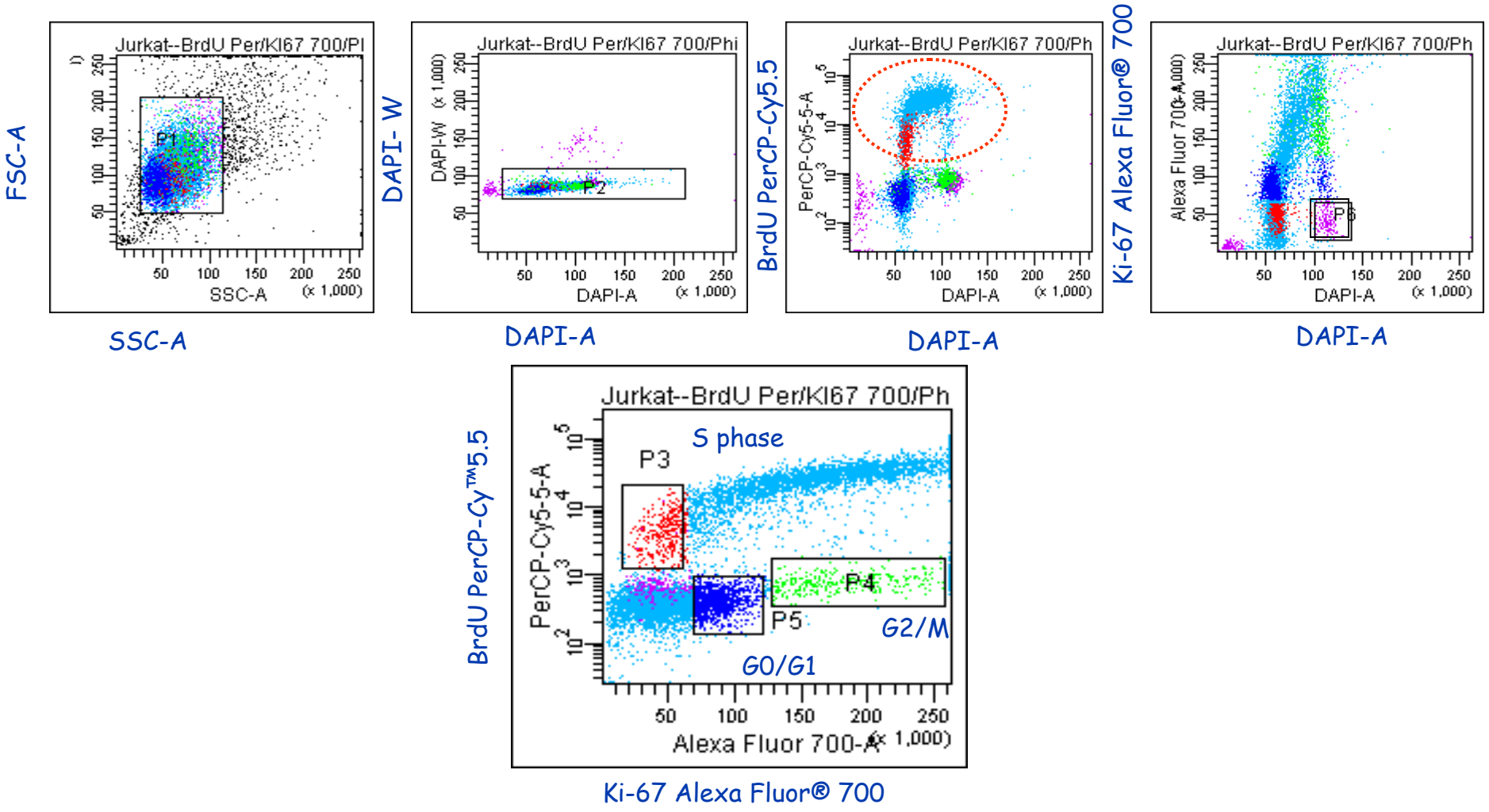


Experimental design:

Mouse splenocytes were incubated with 1 µM 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.

Ki-67: Another Marker for Cell Proliferation



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

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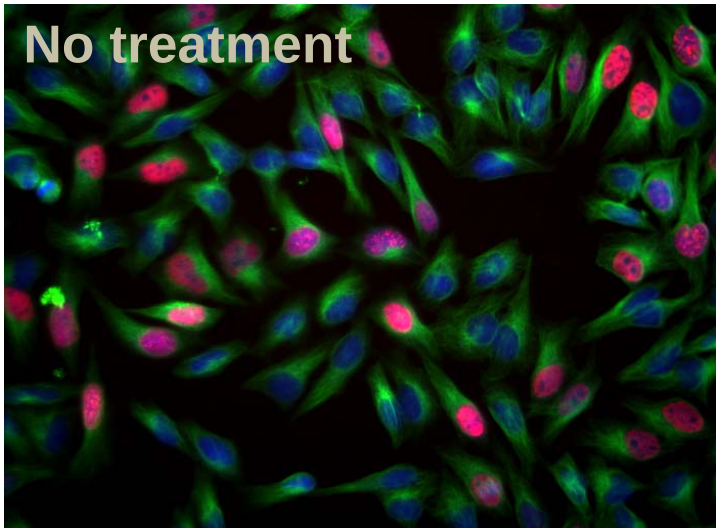
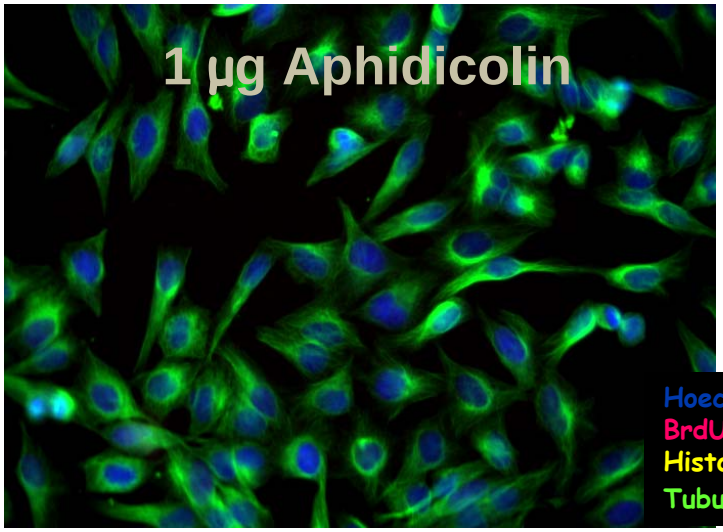
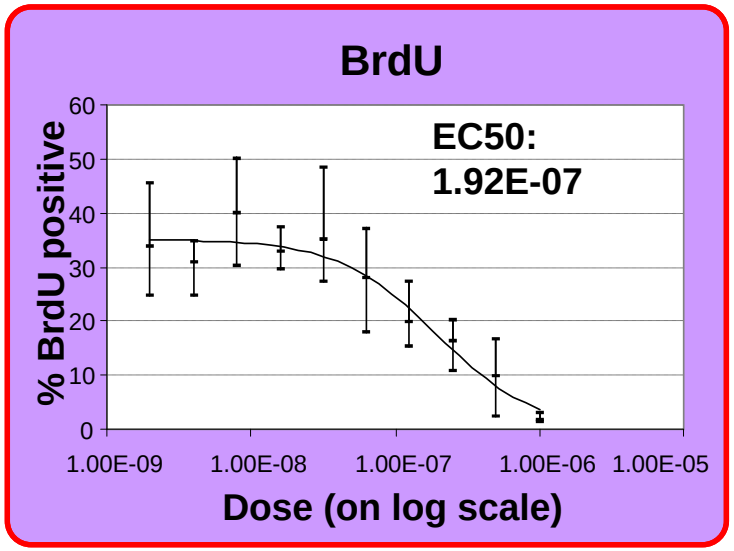
Cell Cycle Analysis on HeLa Cells Treated with Aphidicolin (DNA Topisomerase α Inhibitor) Monitored by BrdU Staining





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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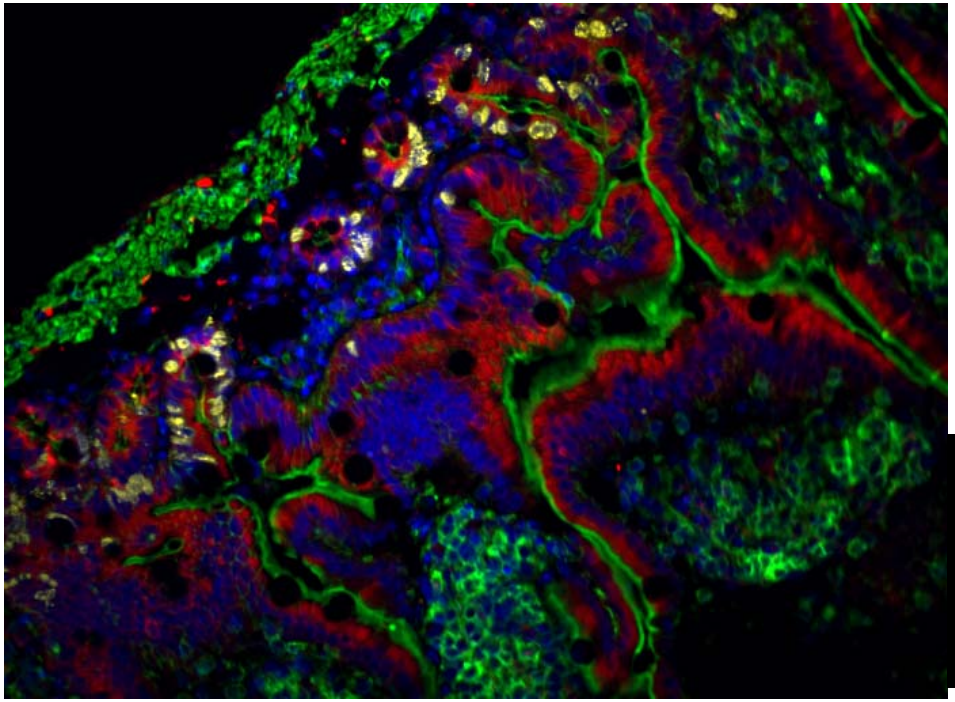
Cell Proliferation Assessed in Mouse Small Intestinal Sections by BrdU Staining



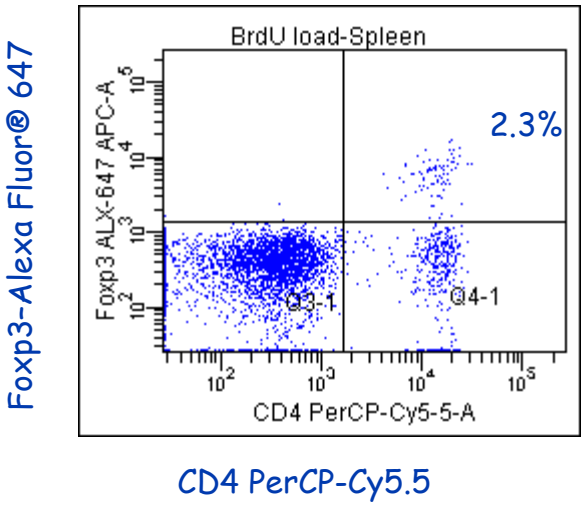
The images were captured on a BD Pathway™ 435 bioimaging system with a 20x objective and merged using BD Attovision software.

Regulatory T Cells (Tregs)

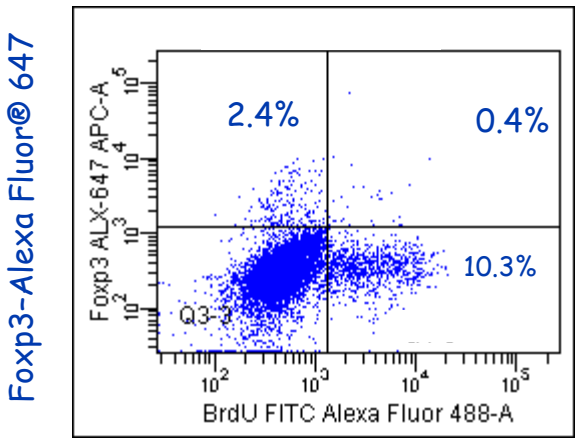
Regulatory T cells, also called "Tregs," play an important role in maintaining immunological unresponsiveness to self antigens (self tolerance) and control of immune responses to foreign antigens.

- 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

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



CD4 PerCP-Cy5.5



BrdU FITC

Experimental design

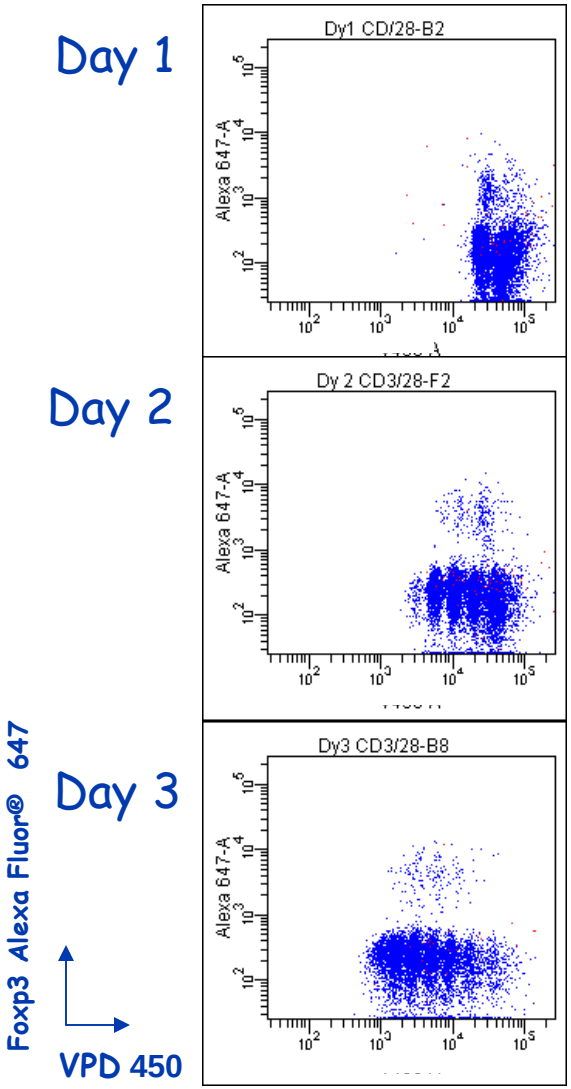
Mouse splenocytes were stimulated with CD3/CD28 and cultured for 5 days. At the end of 5 days, cells were further cultured and then re-stimulated for 5 hours with PMA+Ionomycin. The cells were incubated with 100 µM BrdU for the final hour of culture and then harvested.

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.

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 µM, 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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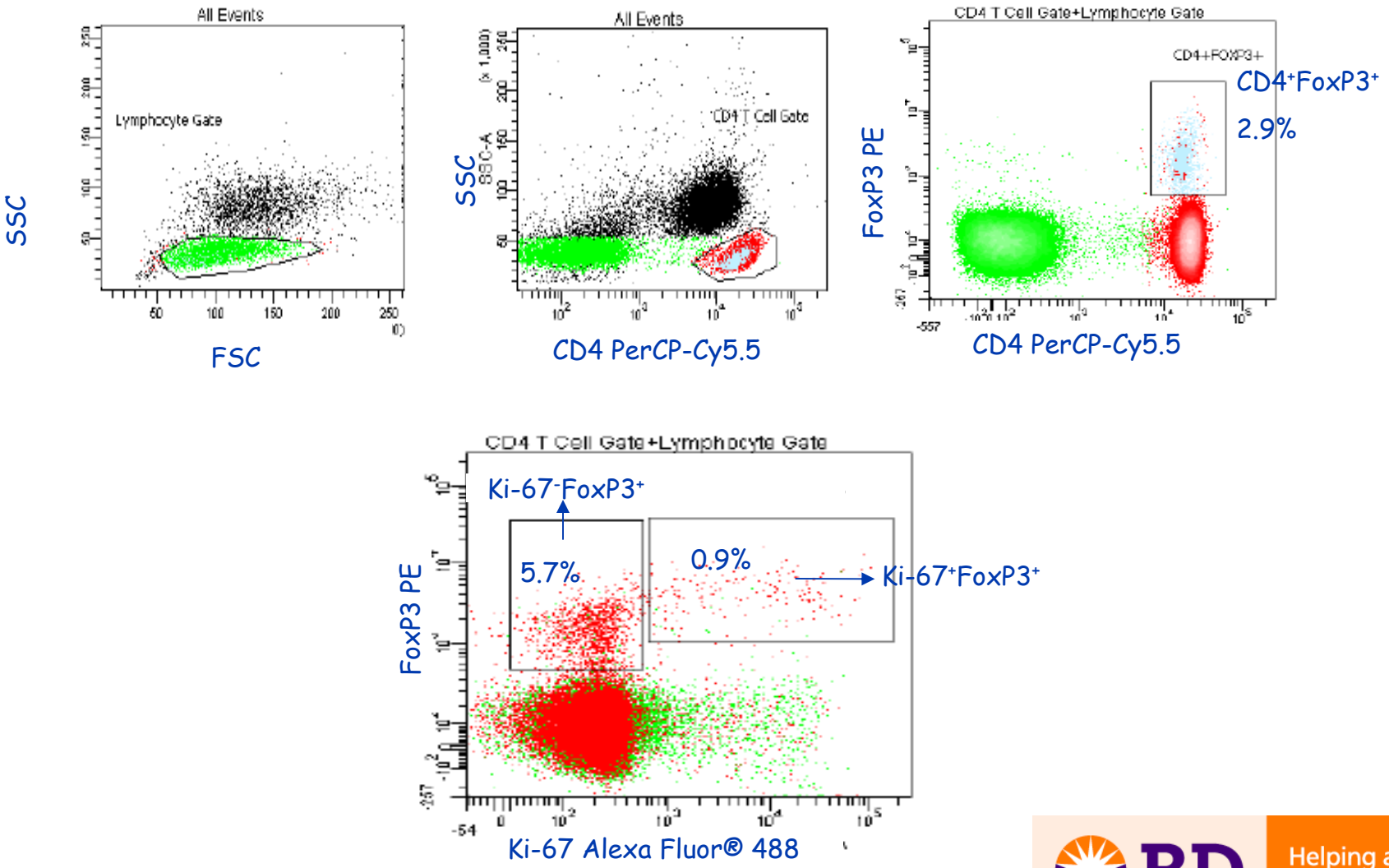
Expression of Ki-67 in Human Tregs



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

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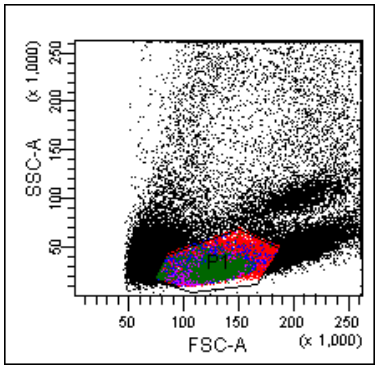
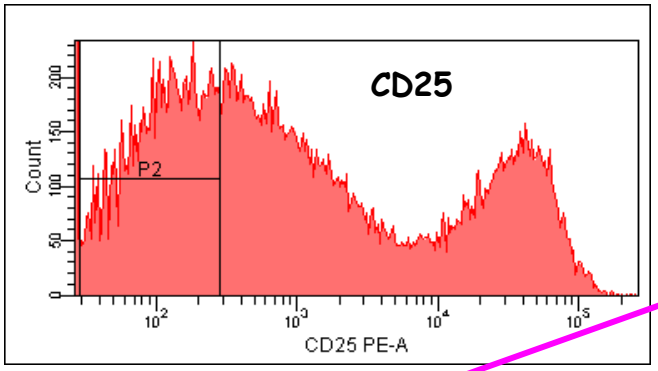
New Assay to Assess Treg Function: Suppression of Effector Cells

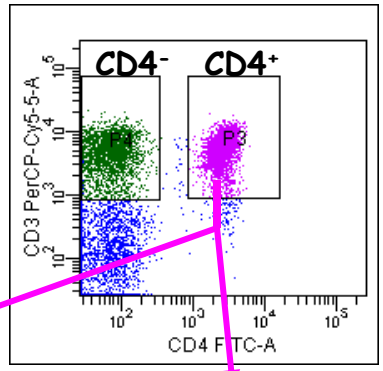
Treg Suppression Assay Kit: how the assay works

- 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.

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





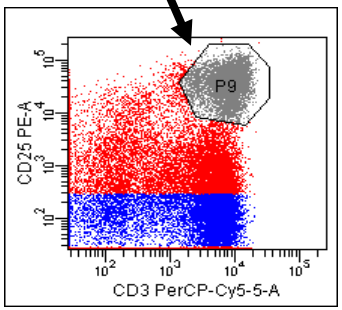


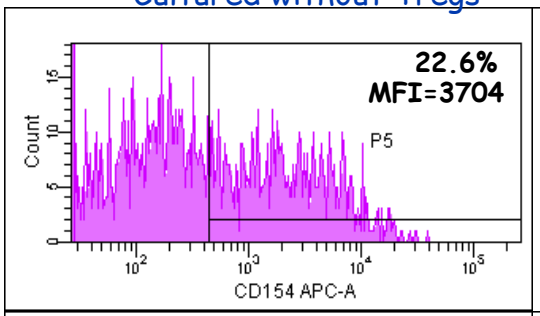
Cultured without Tregs

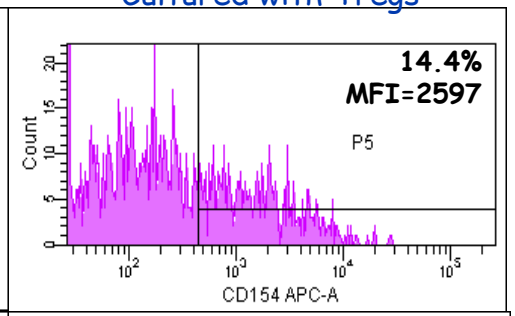
Cultured with Tregs

Tregs

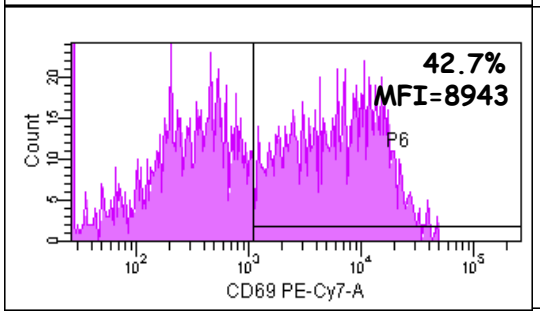
CD154

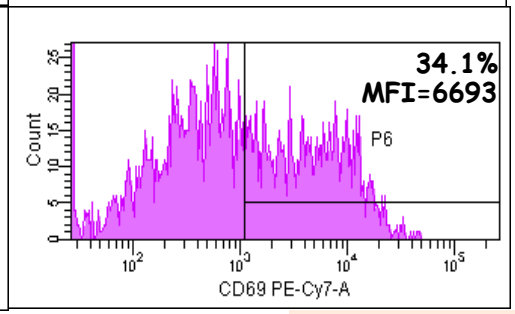






CD154





CD69

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Apoptosis

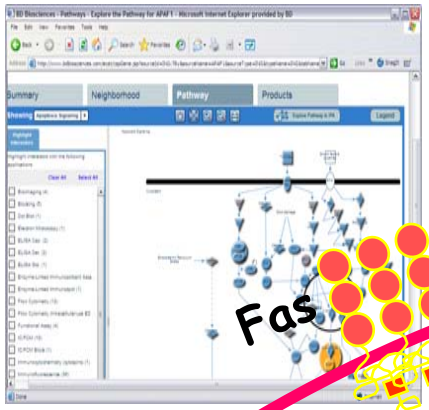
Definition: The process leading to controlled self-destruction of a cell. Cells undergo death neatly without damaging their neighbors. Apoptosis is a "programmed event."

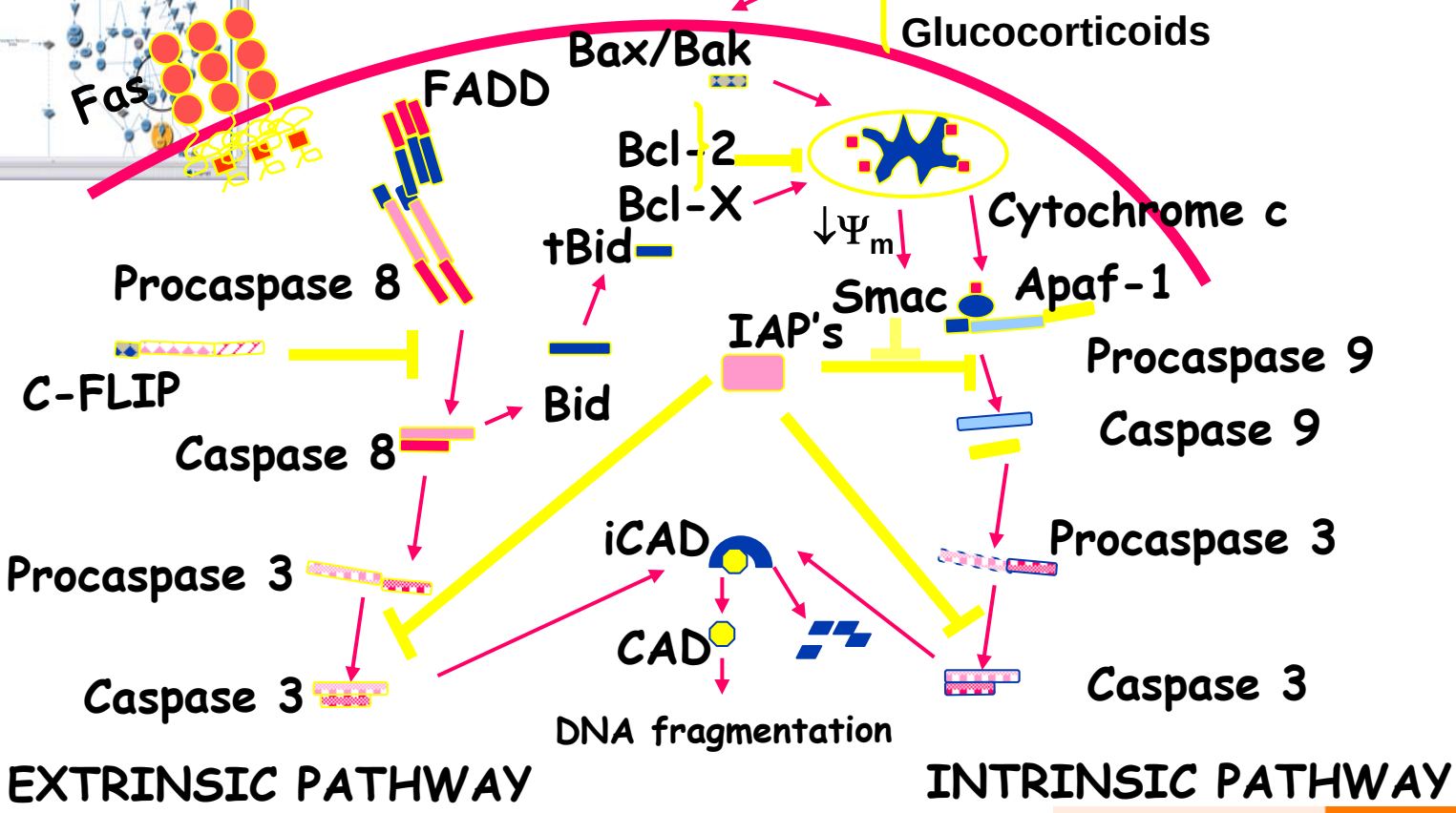
Importance of Apoptosis

- 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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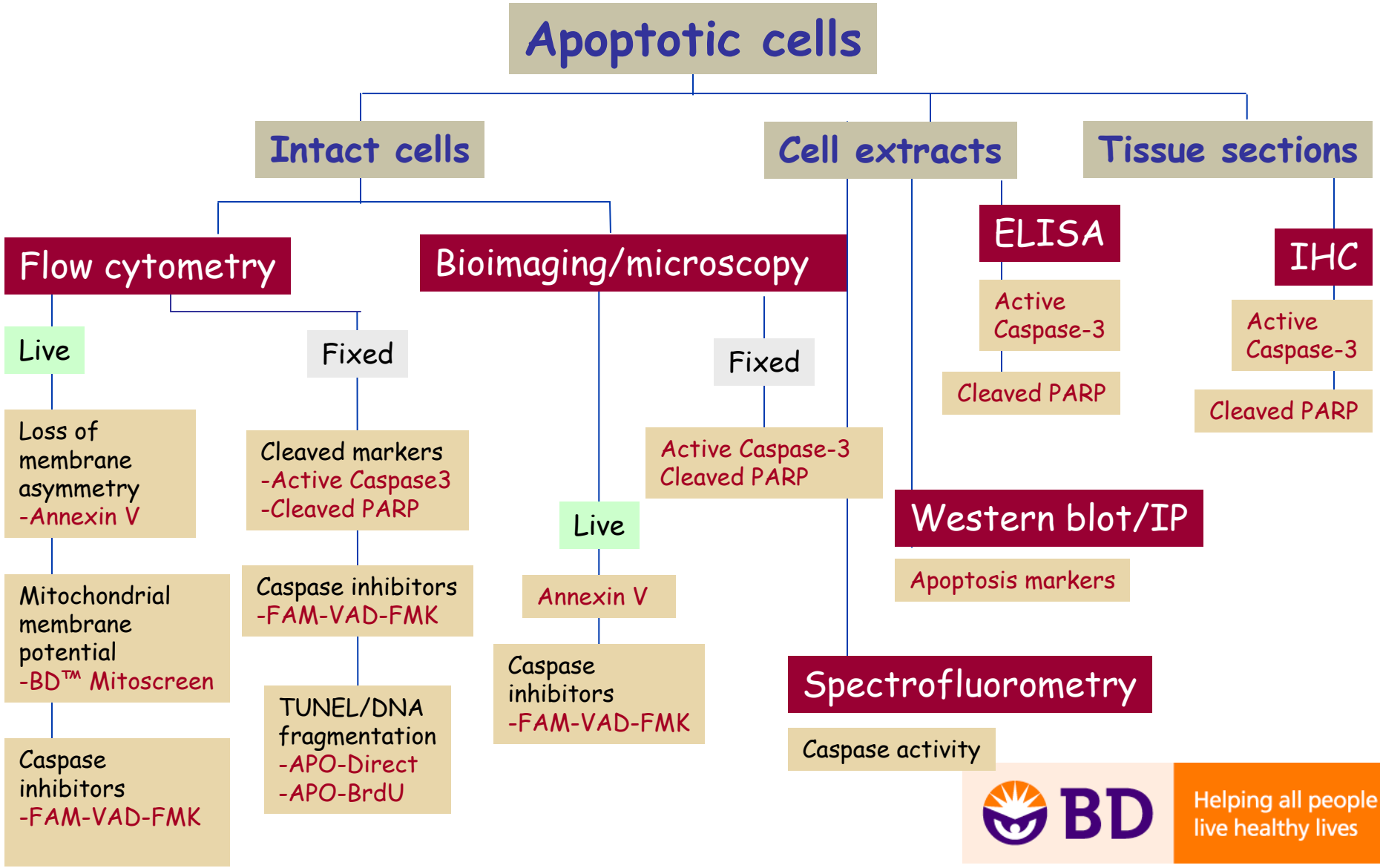
Hallmarks of Apoptosis

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

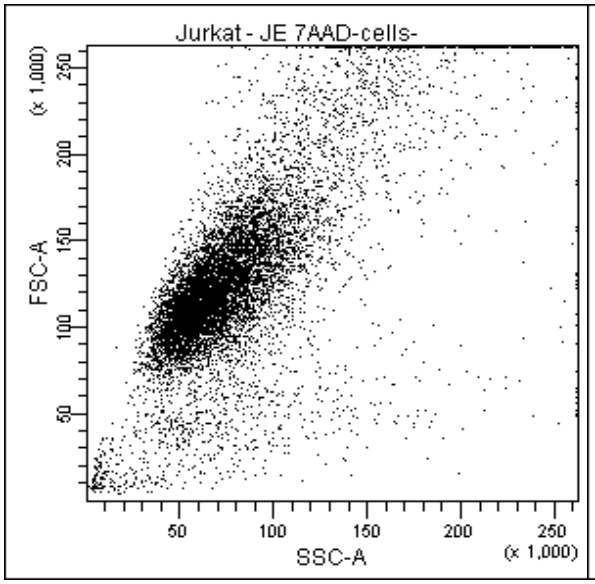
Apoptosis Application Decision Tree



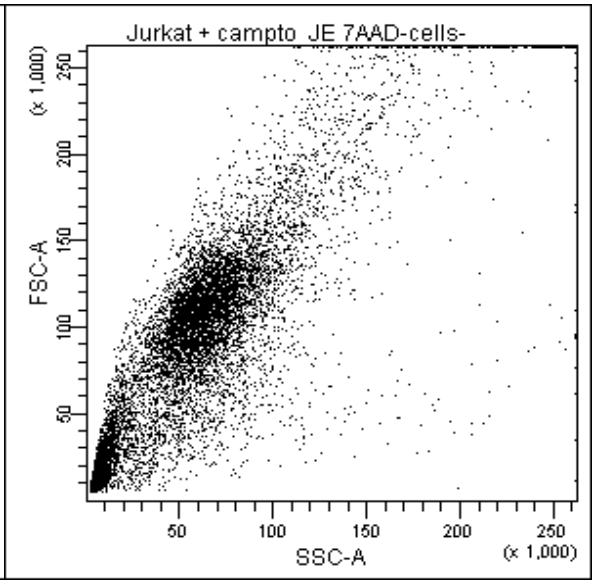
Apoptosis: Scatter Properties

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

Untreated



Camptothecin treated



Formation of apoptotic vesicles

- **Increases side scatter**

Reduced refractive index of apoptotic cells

- **Decreases forward scatter**

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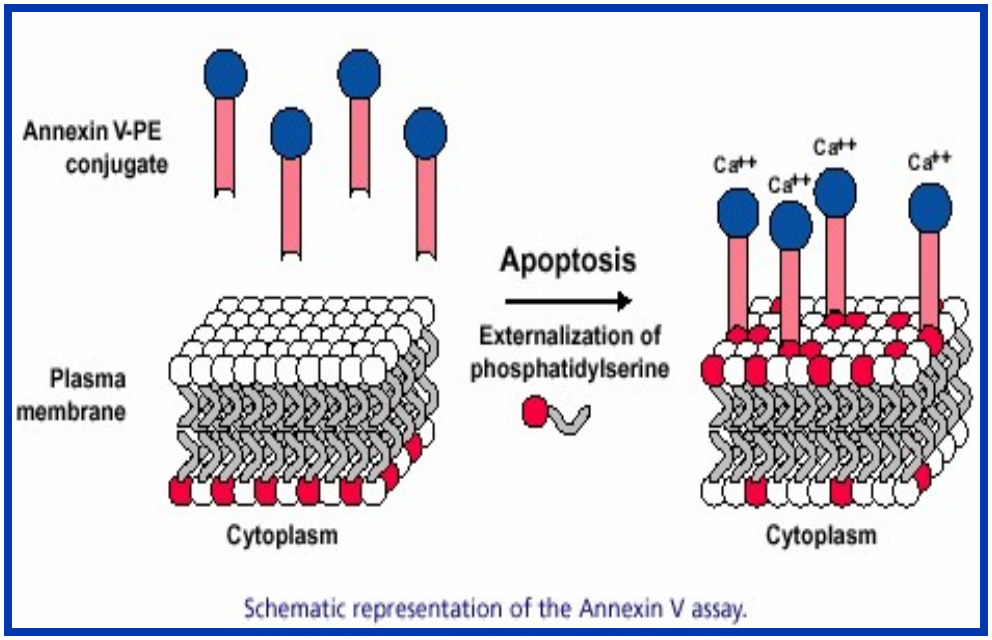
Annexin V

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)



Schematic representation of the Annexin V assay.

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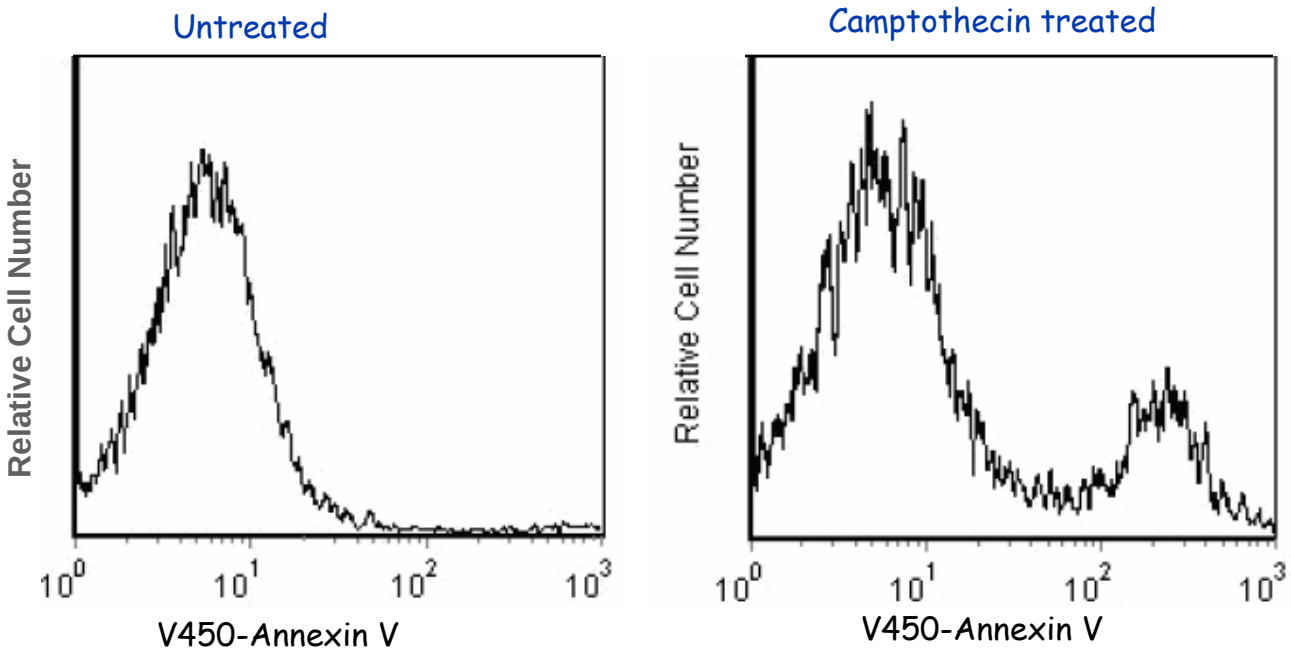
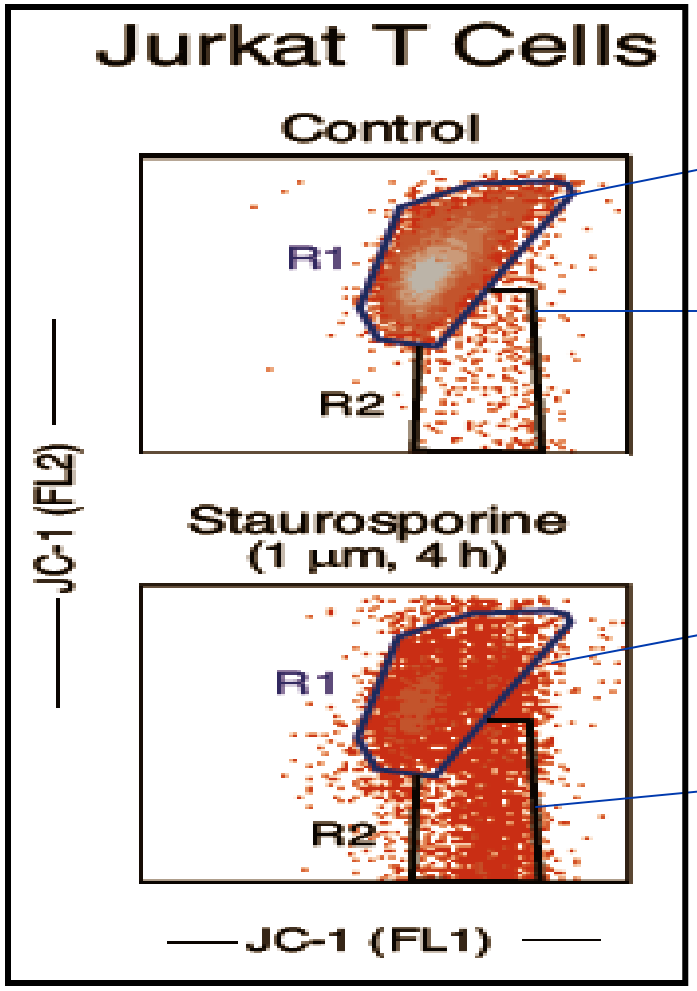
Detection of Membrane Changes by Annexin V Staining and Analysis by Flow Cytometry



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

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

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



J-aggregates (healthy cell)

monomers (apoptosis indicator)

J-aggregates (healthy cell)

monomers (apoptosis indicator)

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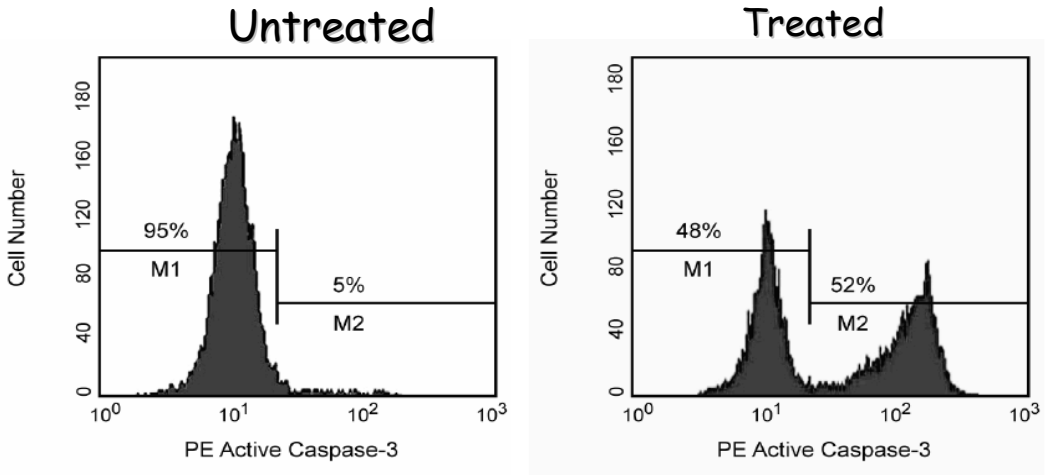
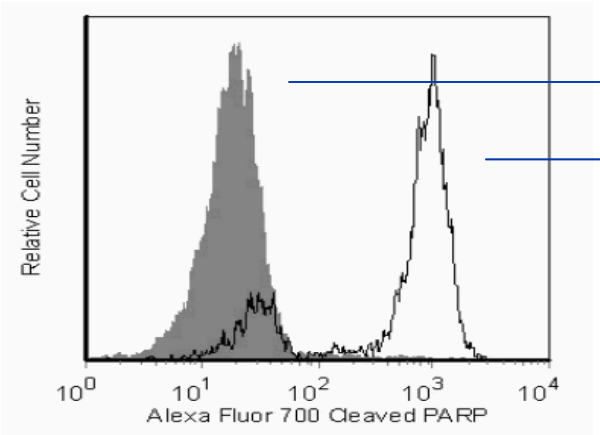
Detection of Active Caspase 3: "Executioner" of Apoptosis and its By-product: Cleaved PARP

Caspase-3



Cleaved-PARP



Untreated/control

Camptothecin treated (6 µM)

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

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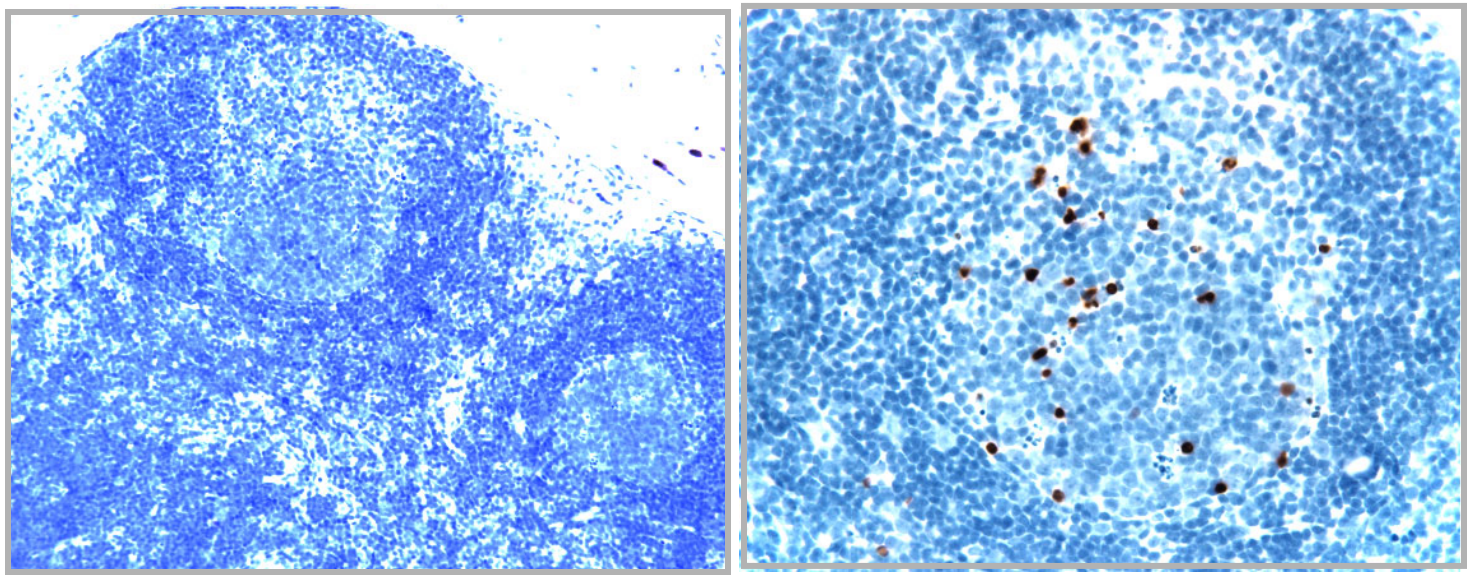
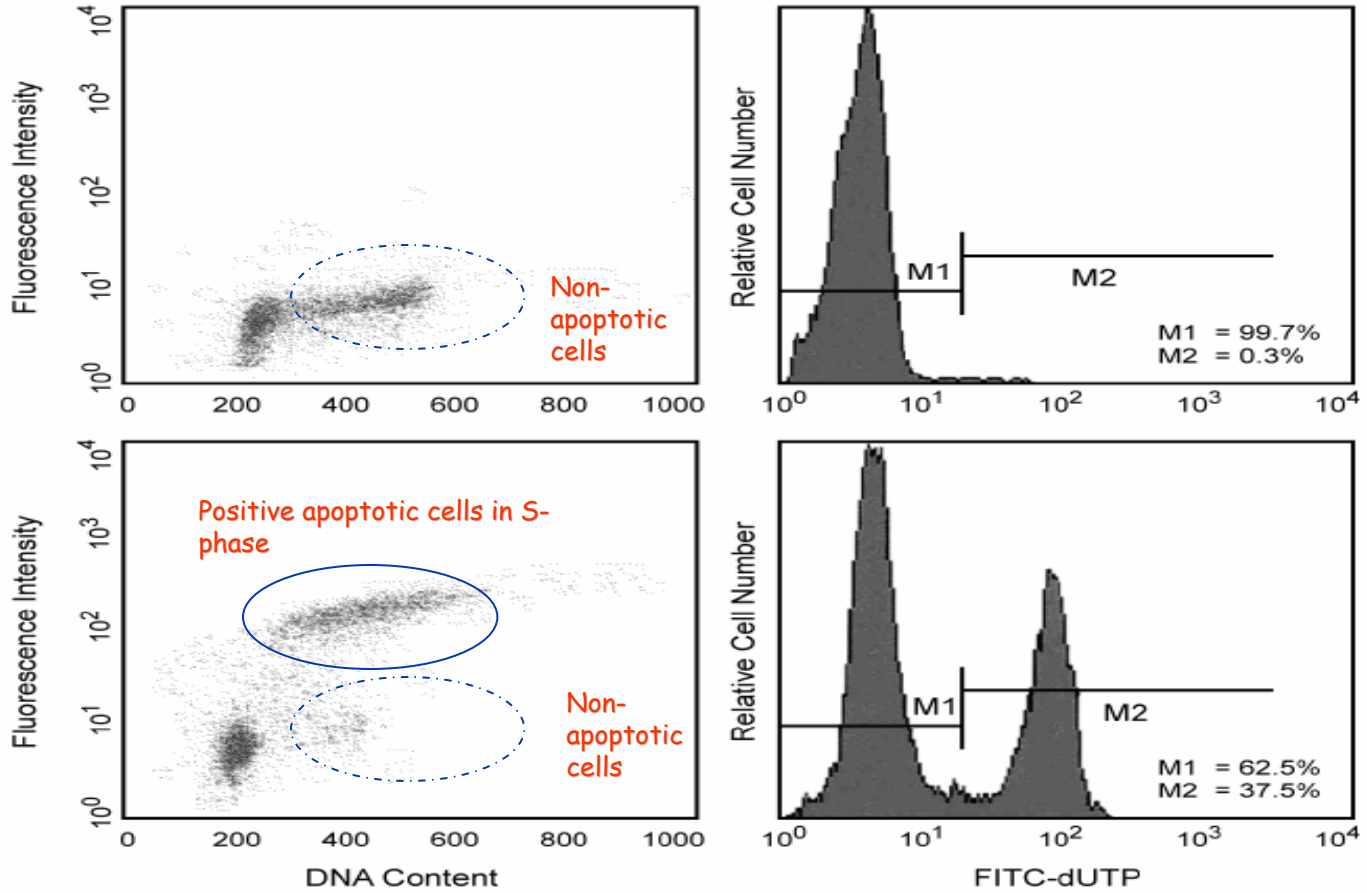
Cleaved PARP Expression in Formalin-fixed, Paraffin-embedded Rat Lymph Node



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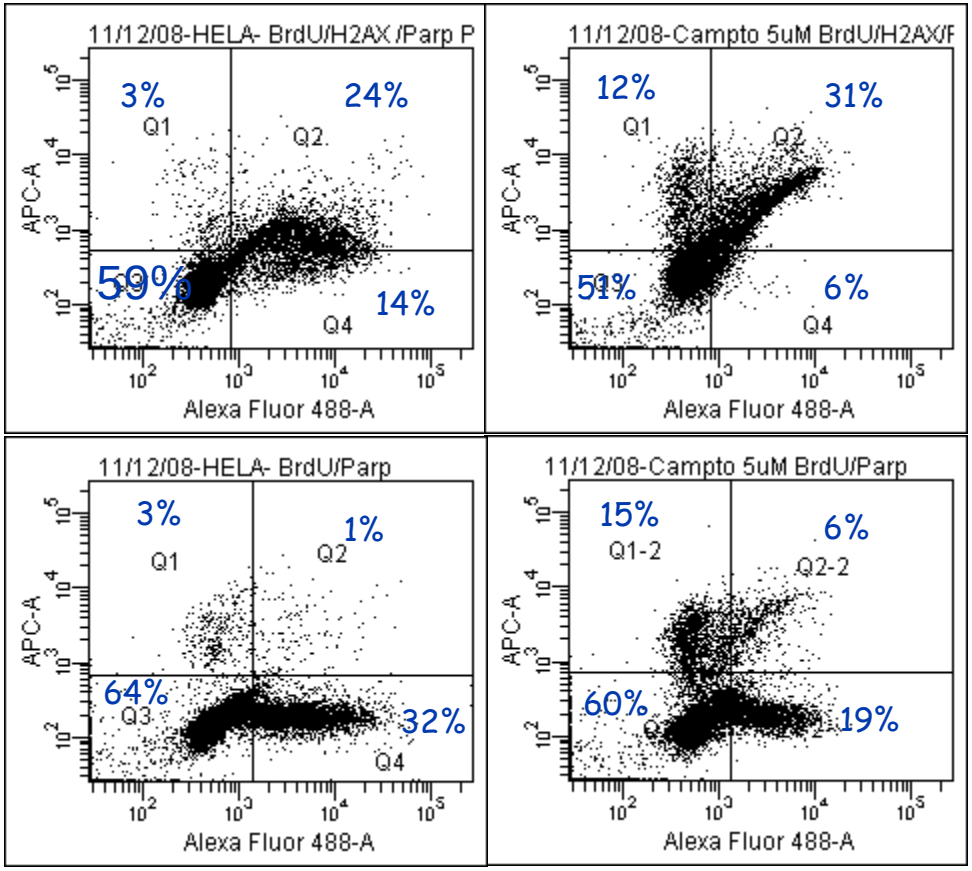
Significant Tool in Drug Discovery Research: Assessing Cell Proliferation, DNA Damage, and Apoptosis Using Flow Cytometry

Untreated

Camptothecin treatment, 5 µM



Phos H2AX-APC

Cleaved PARP-APC

BrdU Alexa Fluor® 488

Experimental design

HeLa cells were untreated or treated with camptothecin 5 µM 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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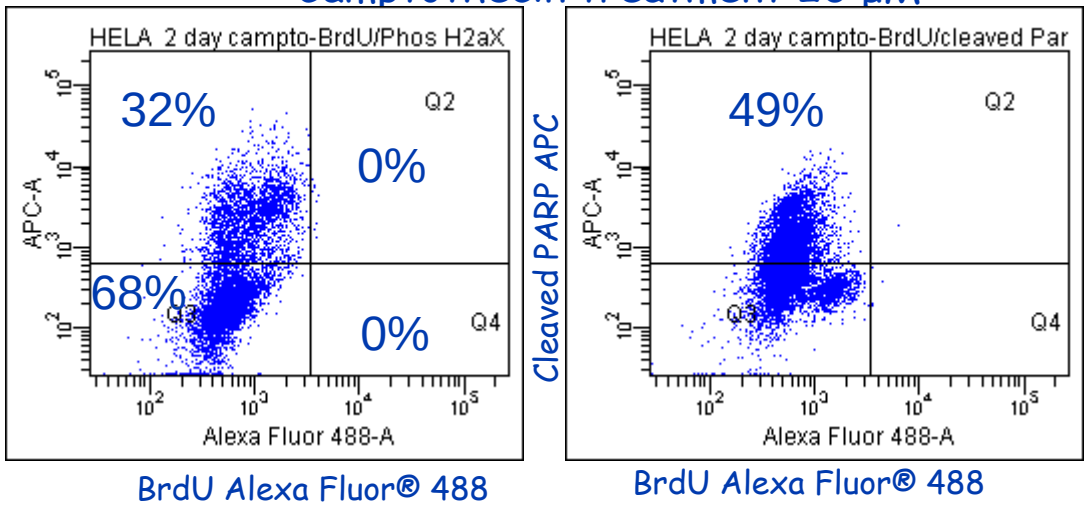
Increase in Camptothecin Concentration and Incubation Time Leads to Increased H2AX and PARP Expression and Loss of BrdU Incorporation

Camptothecin treatment 20 µM



Experimental design

HeLa cells were untreated or treated with camptothecin, 20 µM, 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.

Acknowledgments

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