Flow Cytometry Applications in Cell Biology and Cancer Research

Nil Emre, PhD
BD Biosciences
Research and Development
San Diego, California

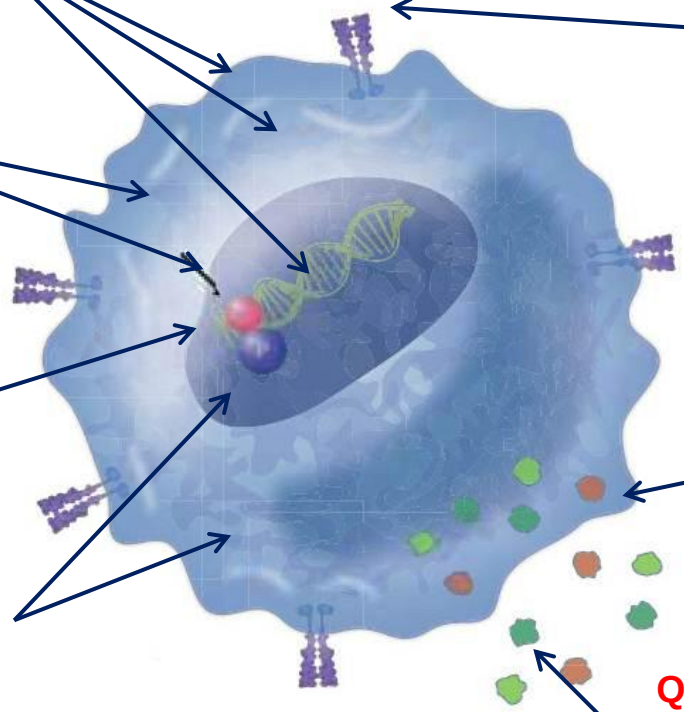
Cell Analysis Using Flow Cytometry

Apoptosis and Cell Viability

Proliferation

Intranuclear
Transcription factors

Intracytoplasmatic or Intranuclear
Cell signaling proteins



Cell Surface
Lineage marker
Activation marker
Cytokine and chemokine receptors

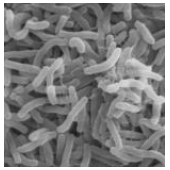
Intracytoplasmatic
Cytokine, chemokines, various proteins

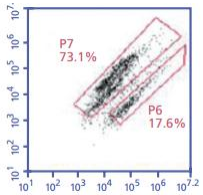
Quantification in supernatant, plasma, serum or cell lysates:
Cytokines, chemokines, immunoglobulins or signaling proteins

Flow Cytometry within Reach™
The BD Accuri™ C6 Personal Flow Cytometry Tour

A Versatile Instrument for Broad Applications

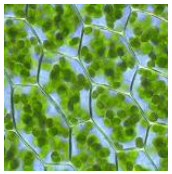
Microbiology

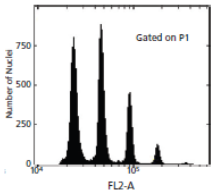




- Aquatic microbiome analysis
- Biofuel research
- Bacteria viability and concentration

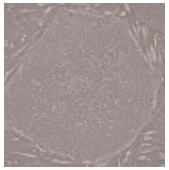
Plant Biology

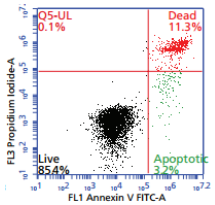




- DNA content

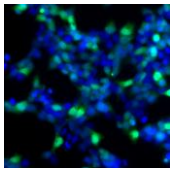
Cell Biology

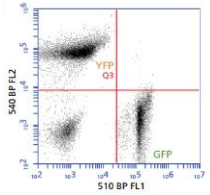




- Apoptosis
- Proliferation
- Immunophenotyping

Fluorescent Protein Analysis





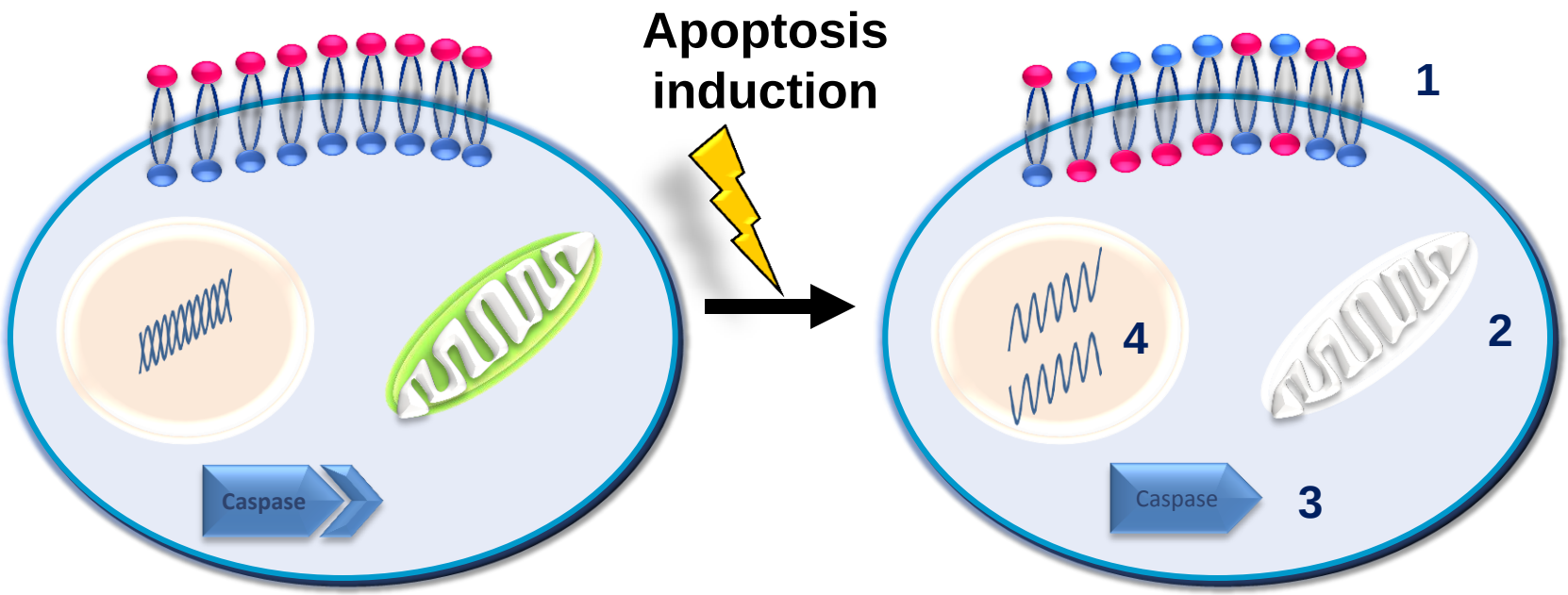
- GFP,YFP
- mCherry, RFP
- mOrange, dTomato

Flow Cytometry Applications in Cell Biology and Cancer Research

- **Cell function assays on flow cytometer**
  - **Apoptosis and viability**
  - **Cell cycle and proliferation**

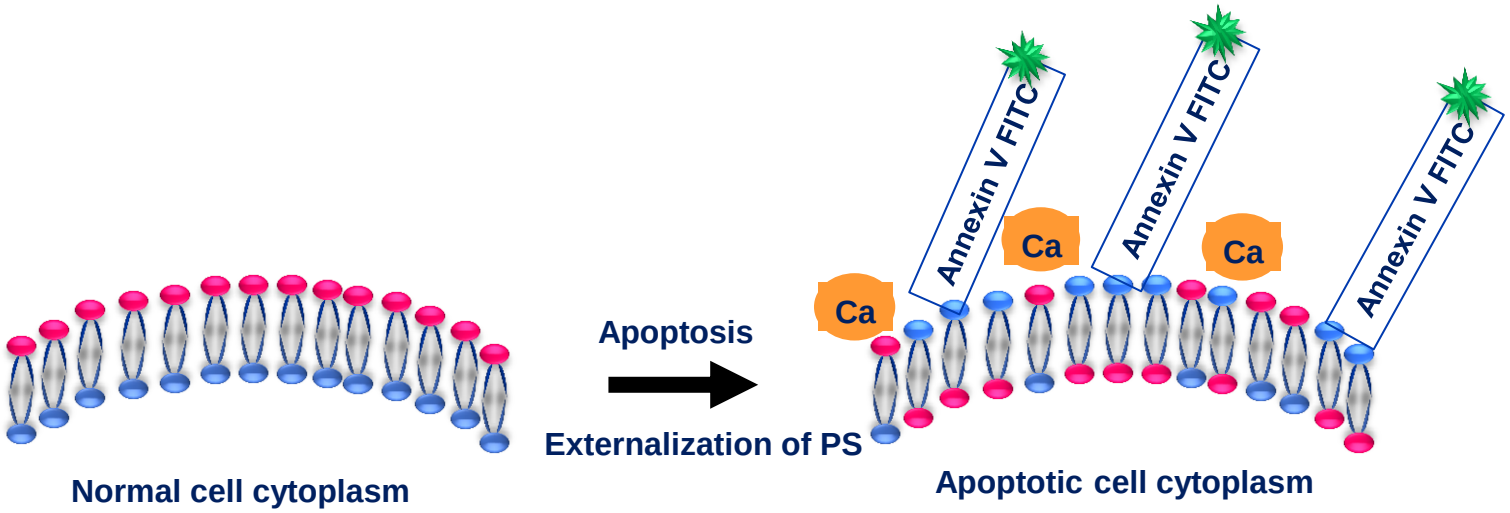
- **Breast cancer cell lines**
  - **Dose-response curves**
  - **Combination cell function assays**
  - **Cell surface signatures**

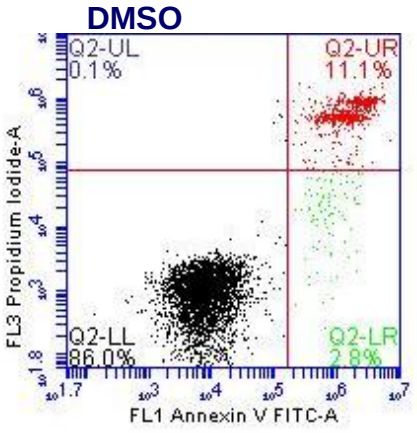
Apoptosis at a Glance

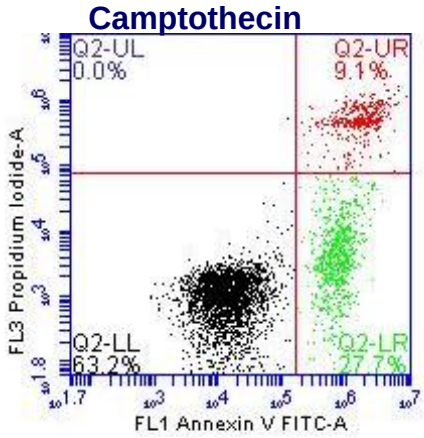


1. Phosphotidyl serine (PS) exposed
2. Mitochondrial potential decreases
3. Caspases activated
4. DNA fragmentation

Apoptosis Membrane Alterations: Annexin V





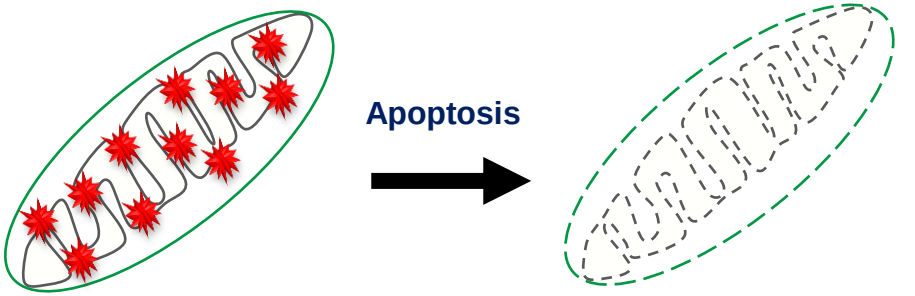


Live cells:
Annexin V Negative
PI Negative

Early apoptotic:
Annexin V Positive
PI Negative

Late apoptotic/dead:
Annexin V Positive
PI Positive

Apoptosis: Mitochondrial Membrane Potential

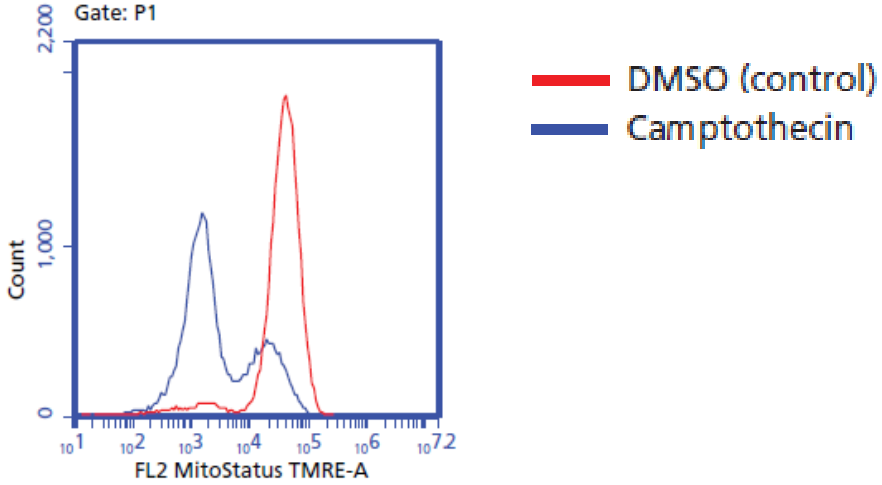


Normal mitochondria

Apoptotic mitochondria

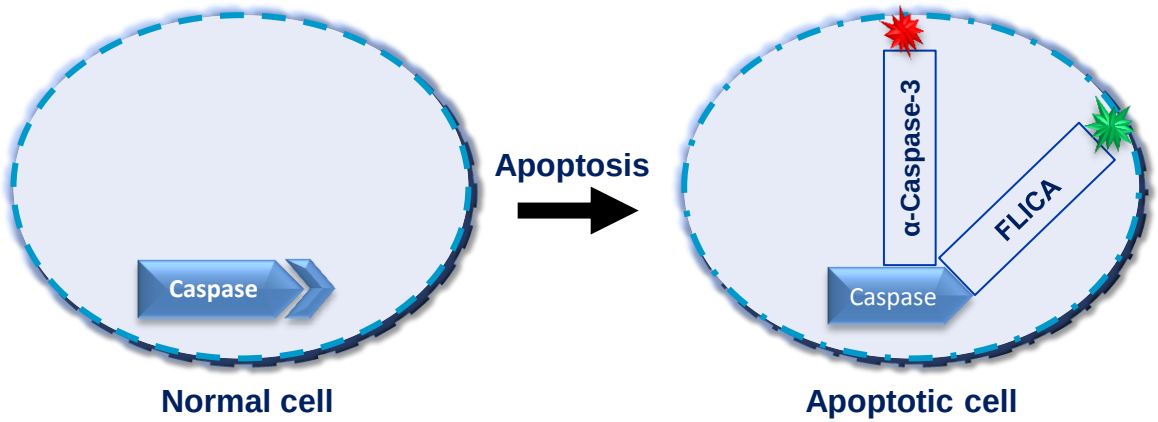
MitoStatus dyes (TMRE and Red)

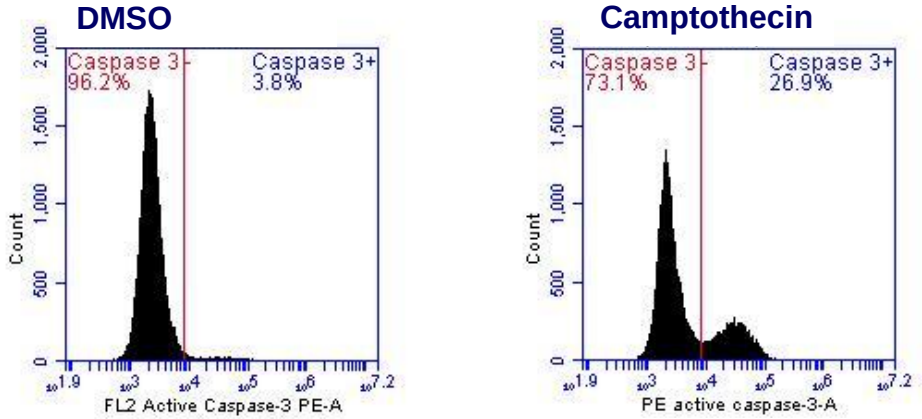
- Healthy cells sequester dye
- Apoptotic cells sequester no dye
- BD Pharmingen™ MitoStatus TMRE (FL2)
- BD Pharmingen™ MitoStatus Red (FL4)



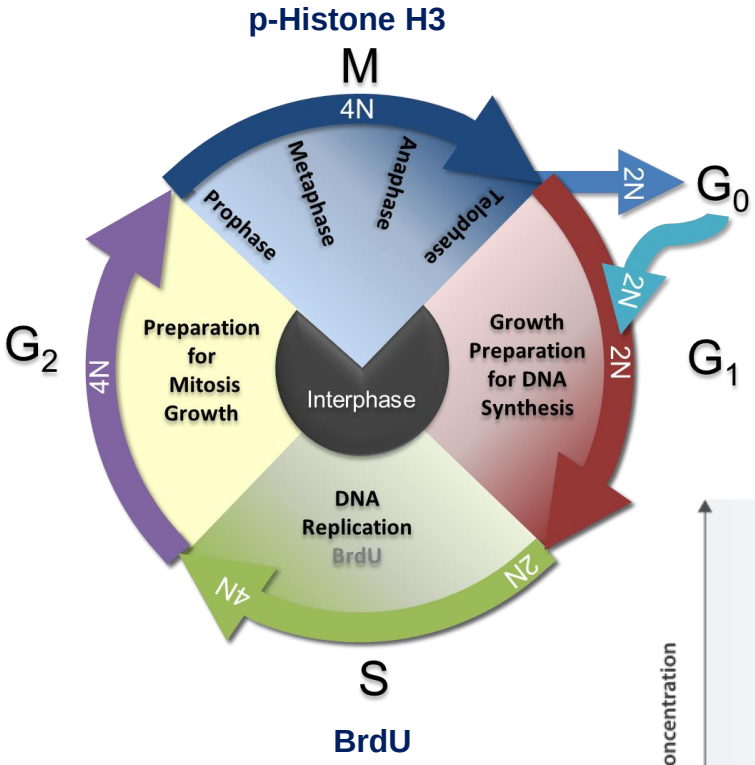
Apoptosis and IC Flow: Active Caspase-3

- **Methods to measure caspase cleavage include fluorogenic inhibitors and detection with antibodies specific to the cleaved (activated) forms of caspases**





Cell Cycle and Proliferation



- DNA content
- Incorporation of BrdU
- Histone phosphorylation
- Expression of specific cyclins

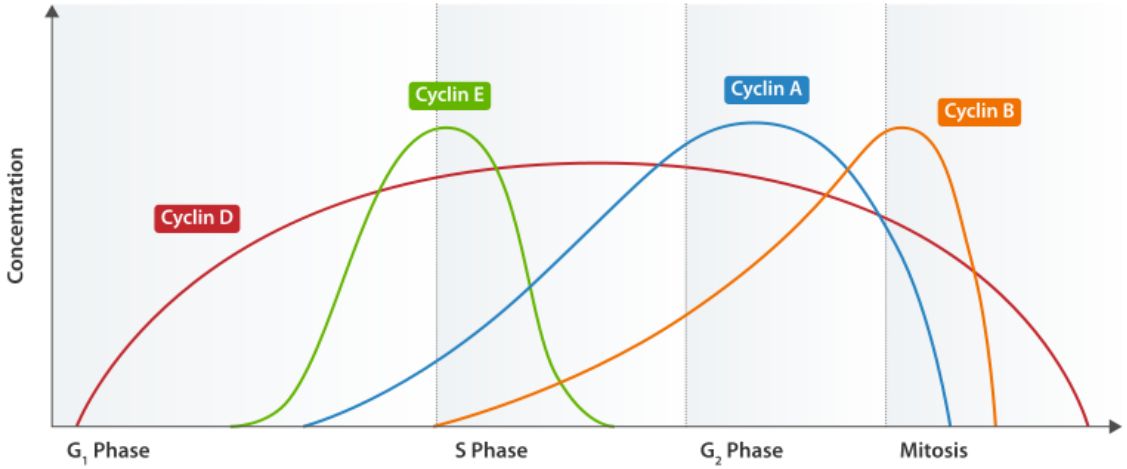
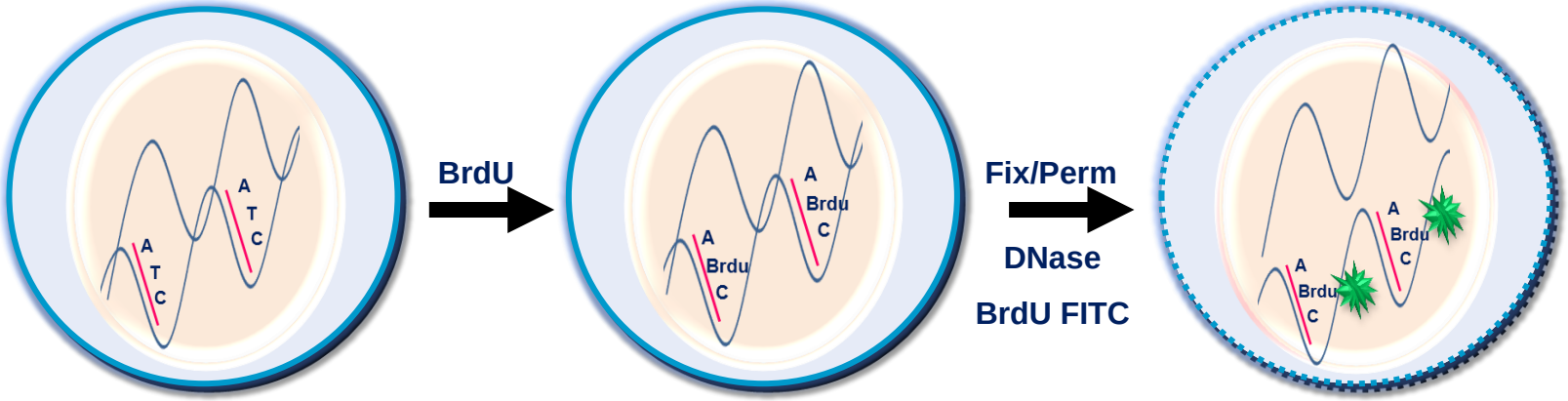
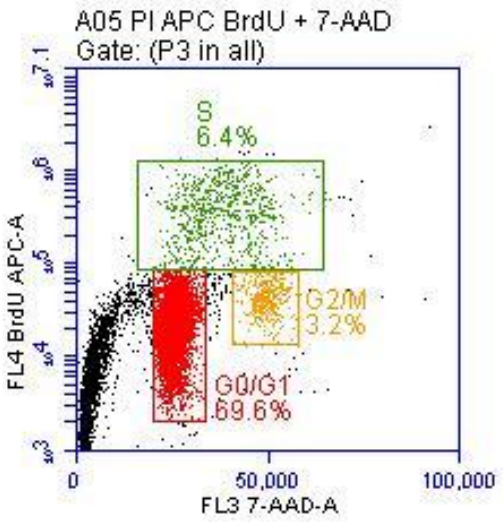


Image Source
http://en.wikipedia.org/wiki/Cyclin

DNA Synthesis: BrdU

- BrdU is incorporated into the DNA of newly synthesized cells (S phase)
- Incorporated BrdU is stained with specific anti-BrdU antibodies
- Staining with a dye that binds total DNA is often coupled with BrdU





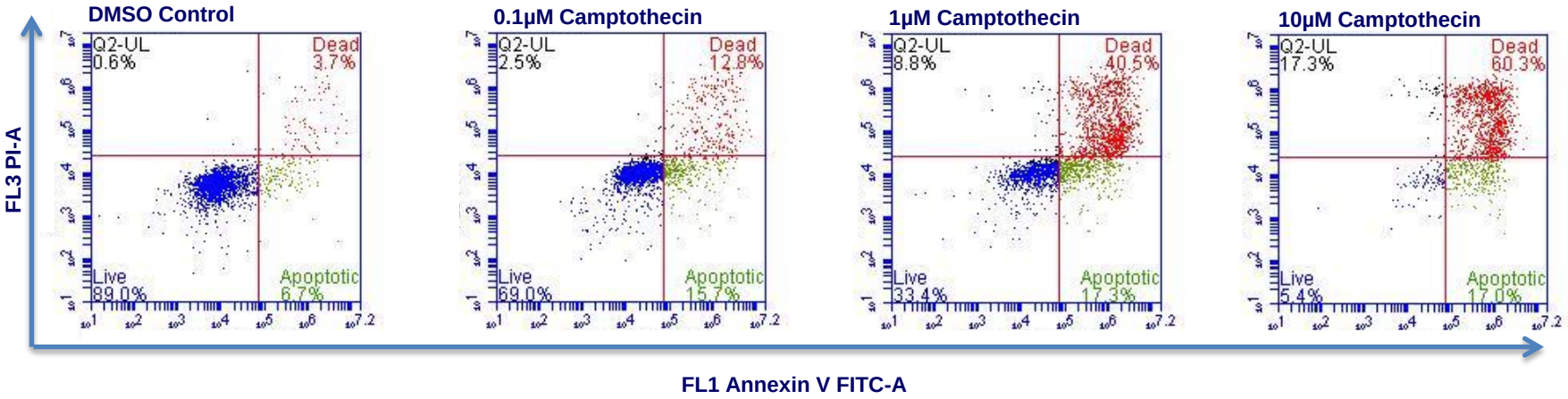
Flow Cytometry Applications in Cell Biology and Cancer Research

- **Breast cancer cell lines:**
  - **MDA-MB-231**
  - **MDA-MB-468**
  - **MCF-7**
- **Cell function assays:**
  - **Dose-response curve**
  - **Multiparameter functional assays**
- **Immunophenotyping:**
  - **Cell surface and intracellular flow cytometry**
  - **Cell surface marker screening**

Flow Cytometry Applications in Cell Biology and Cancer Research

- **Breast cancer cell lines:**
  - **MDA-MB-231**
  - **MDA-MB-468**
  - **MCF-7**
- **Cell function assays:**
  - **Dose-response curve**
  - **Multiparameter functional assays**
- **Immunophenotyping:**
  - **Cell surface and intracellular flow cytometry**
  - **Cell surface marker screening**

MDA-MB-231: Dose Response



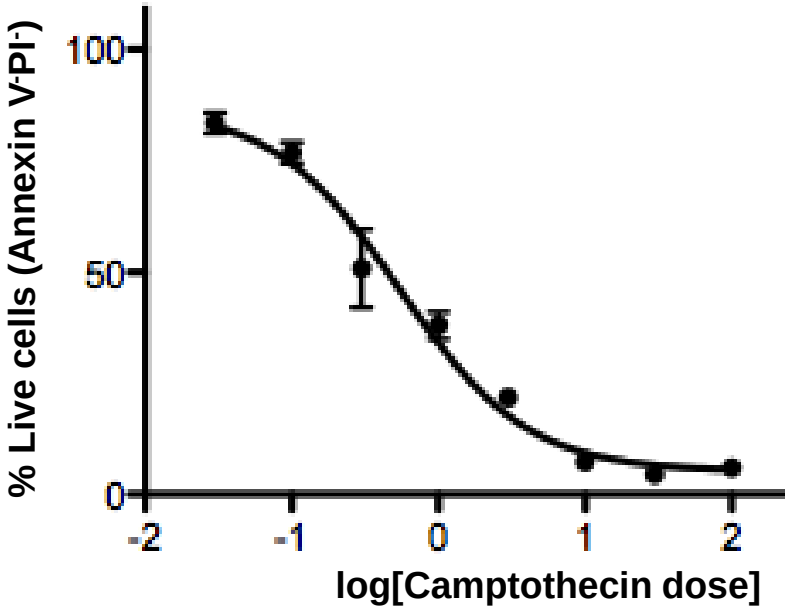
- **MDA-MB-231 cells were treated for 48 hours with varying doses of camptothecin (0.1-100 µM).**
- **Simultaneous measurement of live, apoptotic, and dead cells**

MDA-MB-231: Dose Response

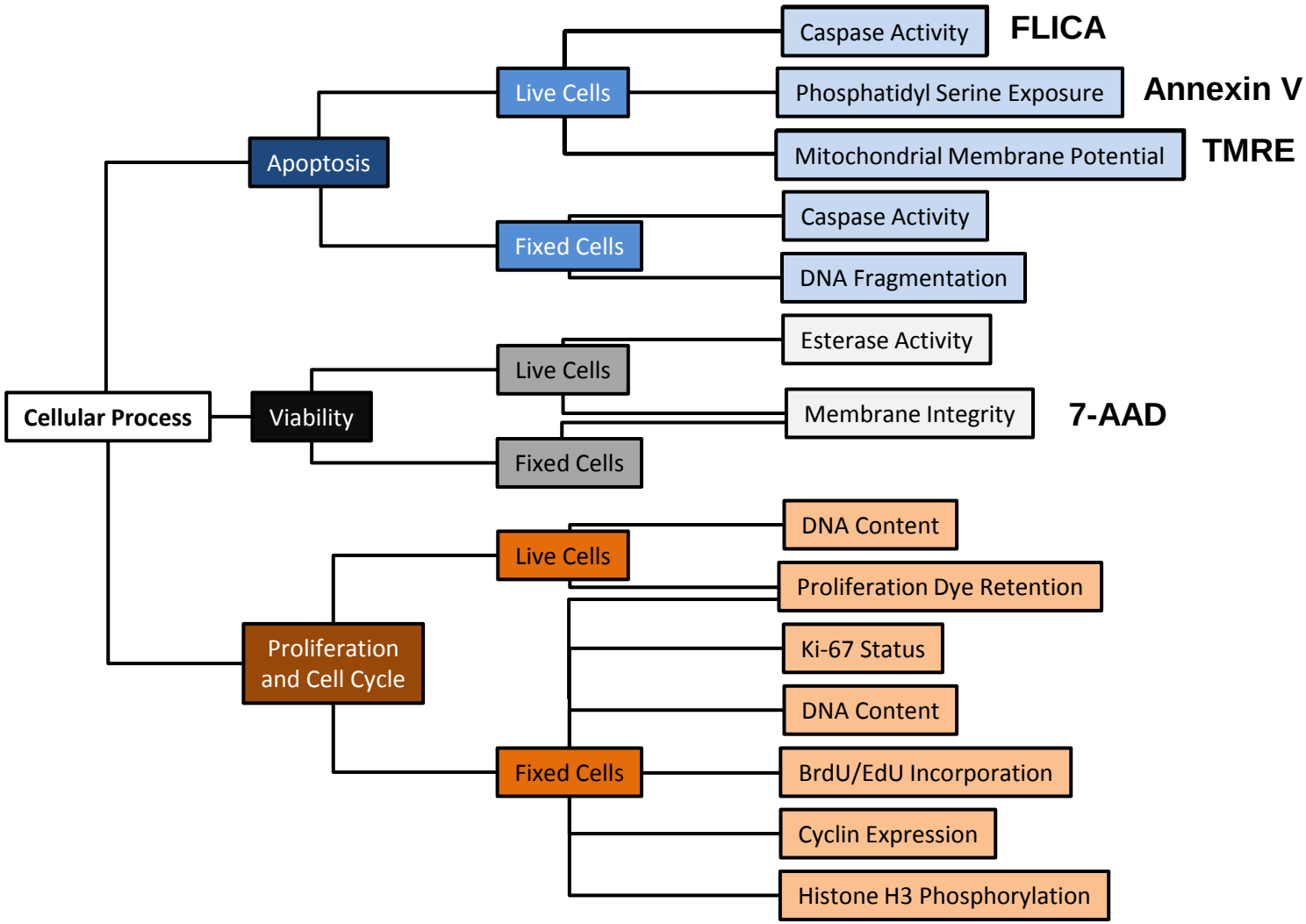
Master Statistics Table

Select cells from the Sample Selector and Statistics Column Selector to build your table.

	Plot 2 (FL1-A/FL3-A)						
	Live		Apoptotic		Dead		This Plot
	Count	% of This Plot	Count	% of This Plot	Count	% of This Plot	Count
A01 100 uM	215	5.78%	320	8.61%	3,024	81.33%	3,718
A02 100 uM	140	5.48%	252	9.87%	2,045	80.07%	2,554
A03 30 uM	75	3.65%	188	9.14%	1,762	85.70%	2,056
A04 30 uM	65	3.40%	222	11.63%	1,592	83.39%	1,909
A05 30 uM	87	5.41%	225	13.99%	1,286	79.98%	1,608
A06 10 uM	126	7.94%	181	11.41%	1,257	79.26%	1,586
A07 10 uM	124	6.40%	197	10.17%	1,576	81.36%	1,937
A08 10 uM	146	6.11%	198	8.28%	1,992	83.35%	2,390
A09 3 uM	736	20.46%	321	8.92%	2,492	69.28%	3,597
A10 3 uM	1,051	19.57%	430	8.01%	3,820	71.12%	5,371
A11 3 uM	958	21.30%	343	7.63%	3,131	69.62%	4,497
A12 1 uM	972	40.93%	200	8.42%	1,160	48.84%	2,375
B01 1 uM	831	30.92%	235	8.74%	1,590	59.15%	2,688
B02 1 uM	1,283	35.56%	331	9.17%	1,926	53.38%	3,608
B03 0.3 uM	569	31.33%	276	15.20%	939	51.71%	1,816
B04 0.3 uM	1,067	56.43%	196	10.36%	590	31.20%	1,891
B05 0.3 uM	1,050	55.70%	218	11.56%	584	30.98%	1,885
B06 0.1 uM	1,276	69.09%	170	9.20%	372	20.14%	1,847
B07 0.1 uM	1,253	71.15%	145	8.23%	347	19.70%	1,761
B08 0.1 uM	1,503	76.57%	158	8.05%	272	13.86%	1,963
B09 0.03 uM	1,613	82.89%	86	4.42%	219	11.25%	1,946
B10 0.03 uM	1,453	76.92%	121	6.41%	283	14.98%	1,889
B11 0.03 uM	1,437	75.95%	140	7.40%	288	15.22%	1,892
B12 0 uM	1,822	91.42%	85	4.26%	79	3.96%	1,993
C01 0 uM	5,481	91.35%	229	3.82%	256	4.27%	6,000
C02 0 uM	2,796	90.78%	103	3.34%	152	4.94%	3,080

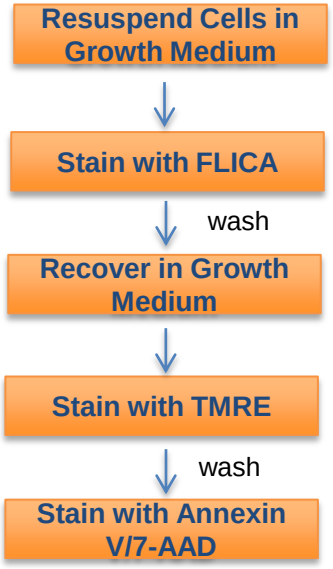


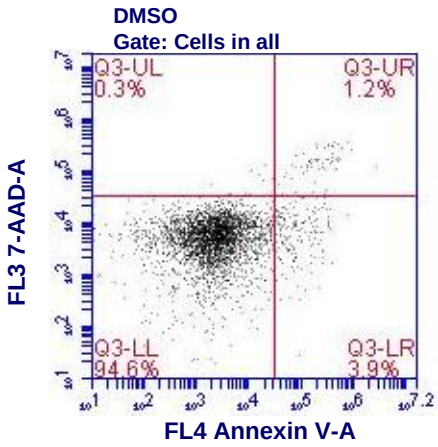
Cell Function Assay Landscape

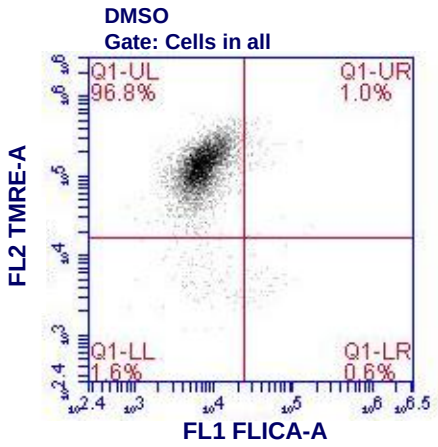


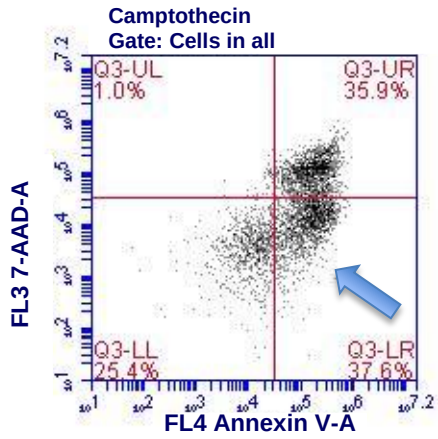
Multiparameter Apoptosis/Viability Analysis on Live Cells: MDA-MB-231

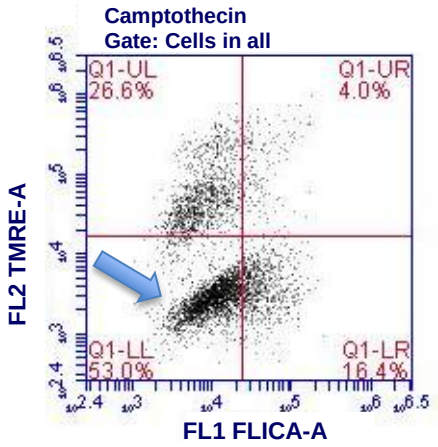
Function	Target	Probe
Apoptosis	Caspase Activity	FLICA (FL1)
Apoptosis	Mitochondrial Membrane Potential	TMRE (FL2)
Viability	Membrane Integrity	7-AAD (FL3)
Apoptosis	Phosphatidyl Serine Exposure	Annexin V (FL4)





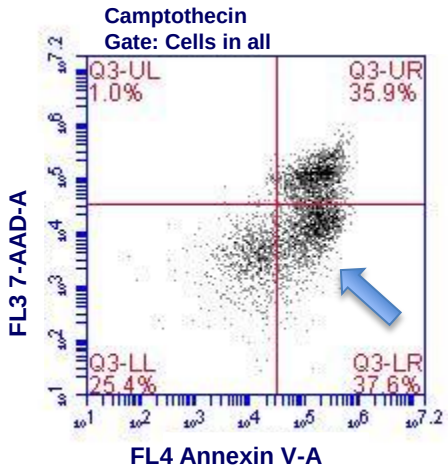


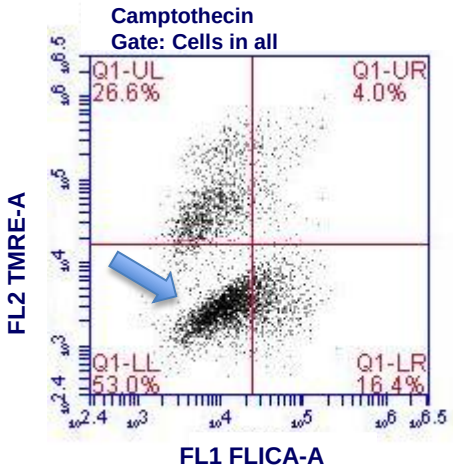




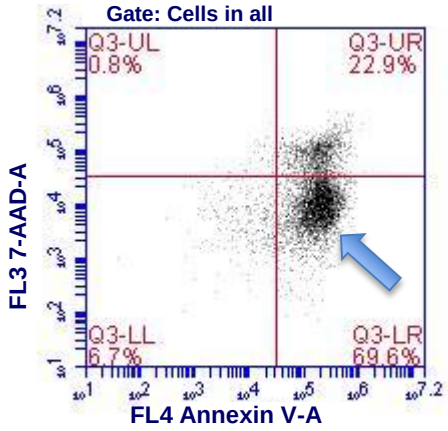
MDA-MB-231, MDA-MB-468: Apoptosis and Cell Viability

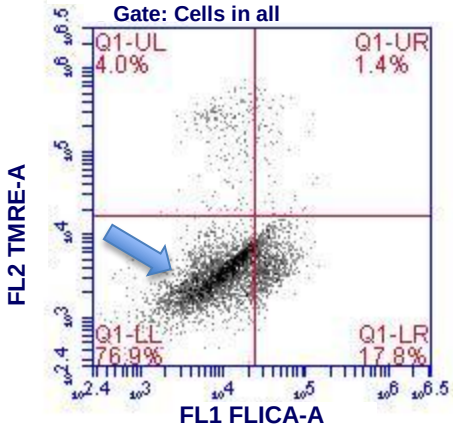
MDA-MB-231





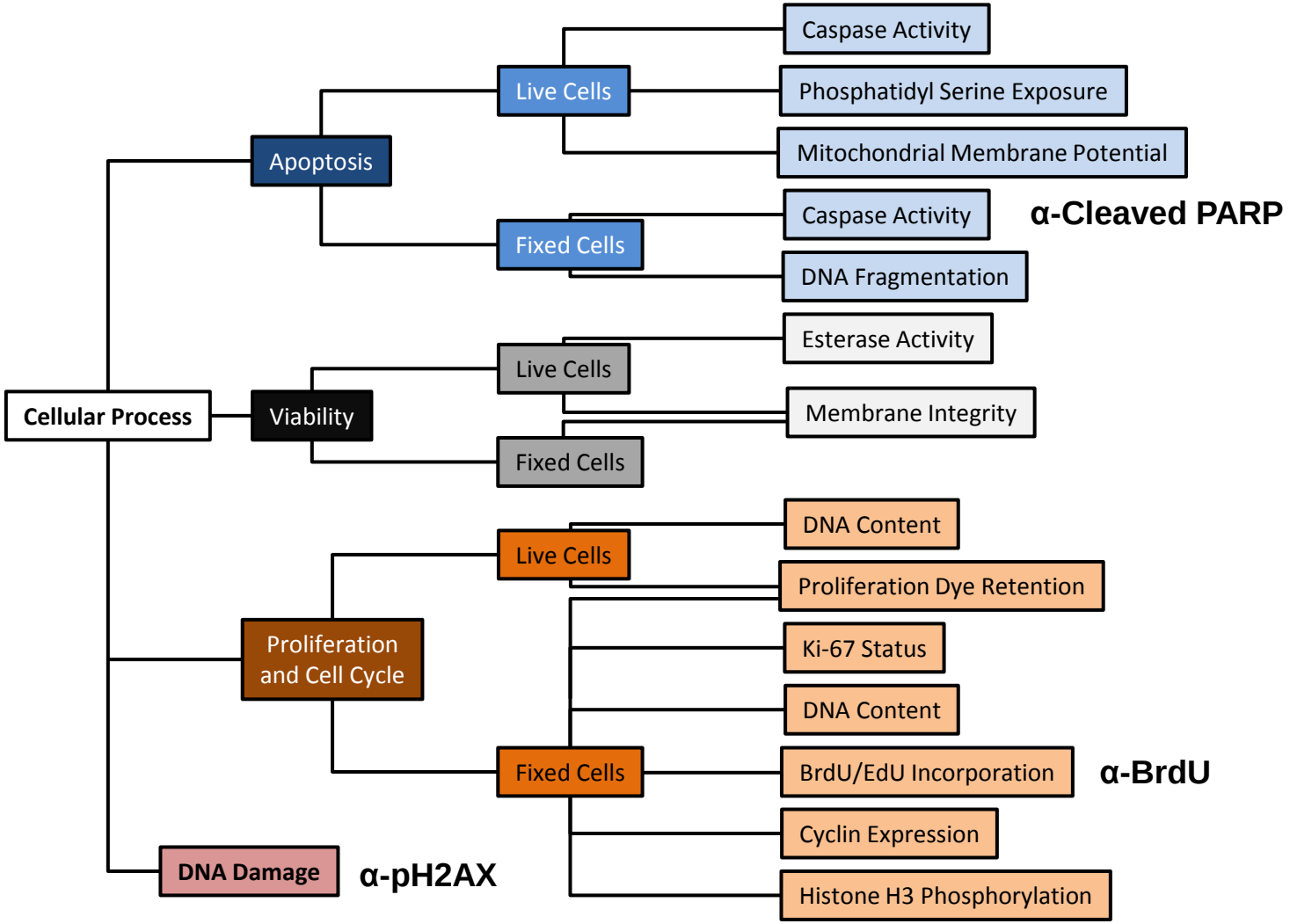
MDA-MB-468





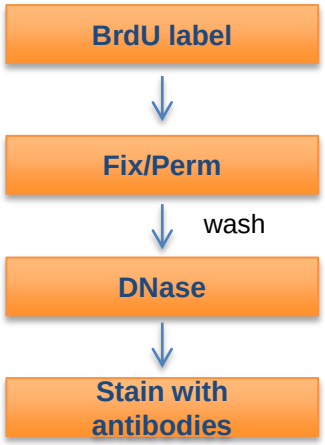
MDA-MB-468 cells have higher levels of phosphatidylserine exposure and membrane depolarization.

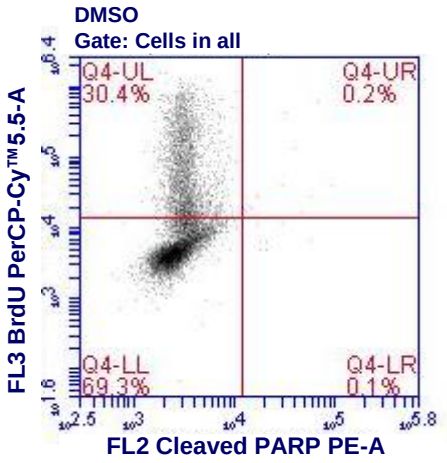
Cell Function Assay Landscape

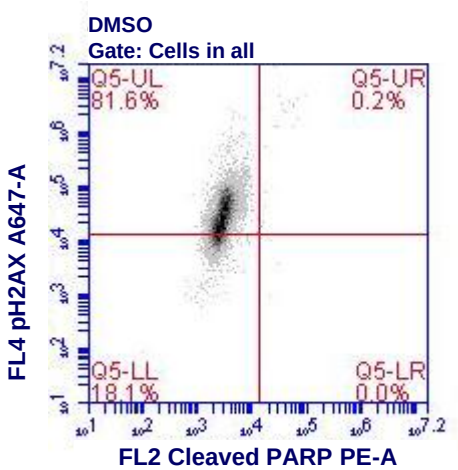


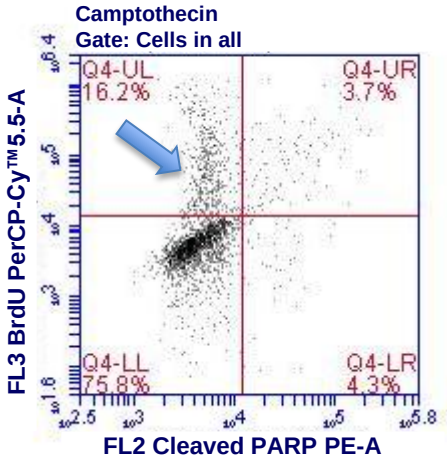
Multiparameter Apoptosis/Proliferation/DNA Damage Analysis on Fixed Cells: MDA-MB-231

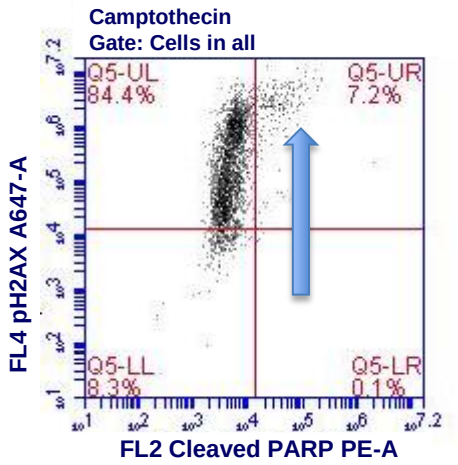
Function	Target	Probe
Apoptosis	Cleaved PARP	α-PARP cleaved form antibody (FL2)
Proliferation	BrdU incorporation	α-BrdU antibody (FL3)
DNA Damage	Phosphorylated H2AX Histone	α-pH2AX (FL4)





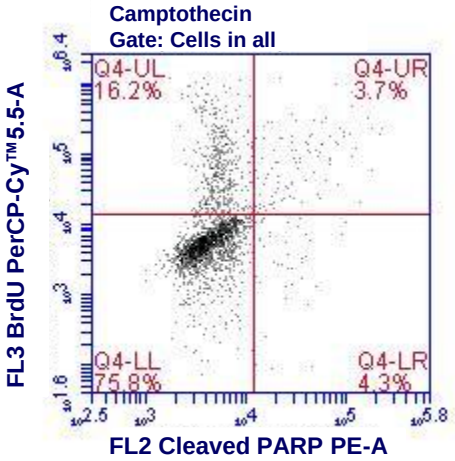


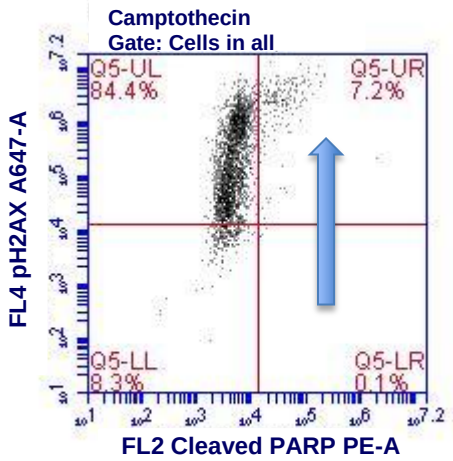




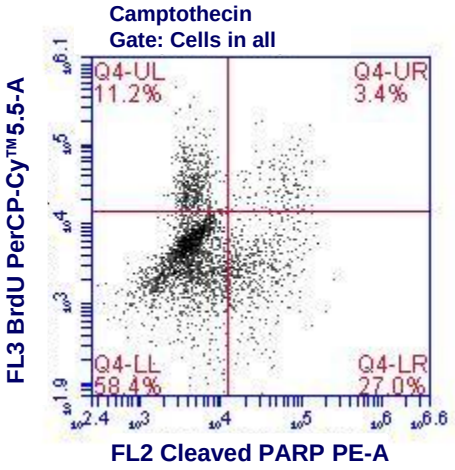
MDA-MB-231, MDA-MB-468: Apoptosis, DNA Damage, and Cell Proliferation

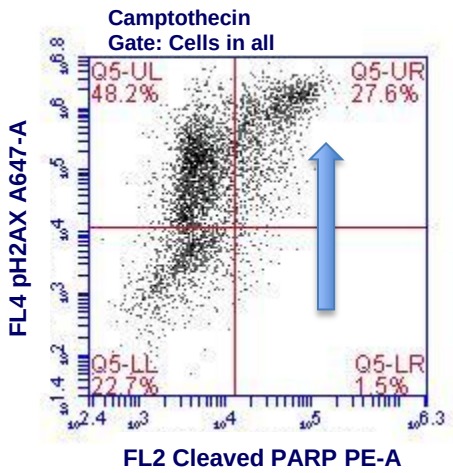
MDA-MB-231





MDA-MB-468





MDA-MB-468 cells have higher levels of Cleaved PARP.

Flow Cytometry Applications in Cell Biology and Cancer Research

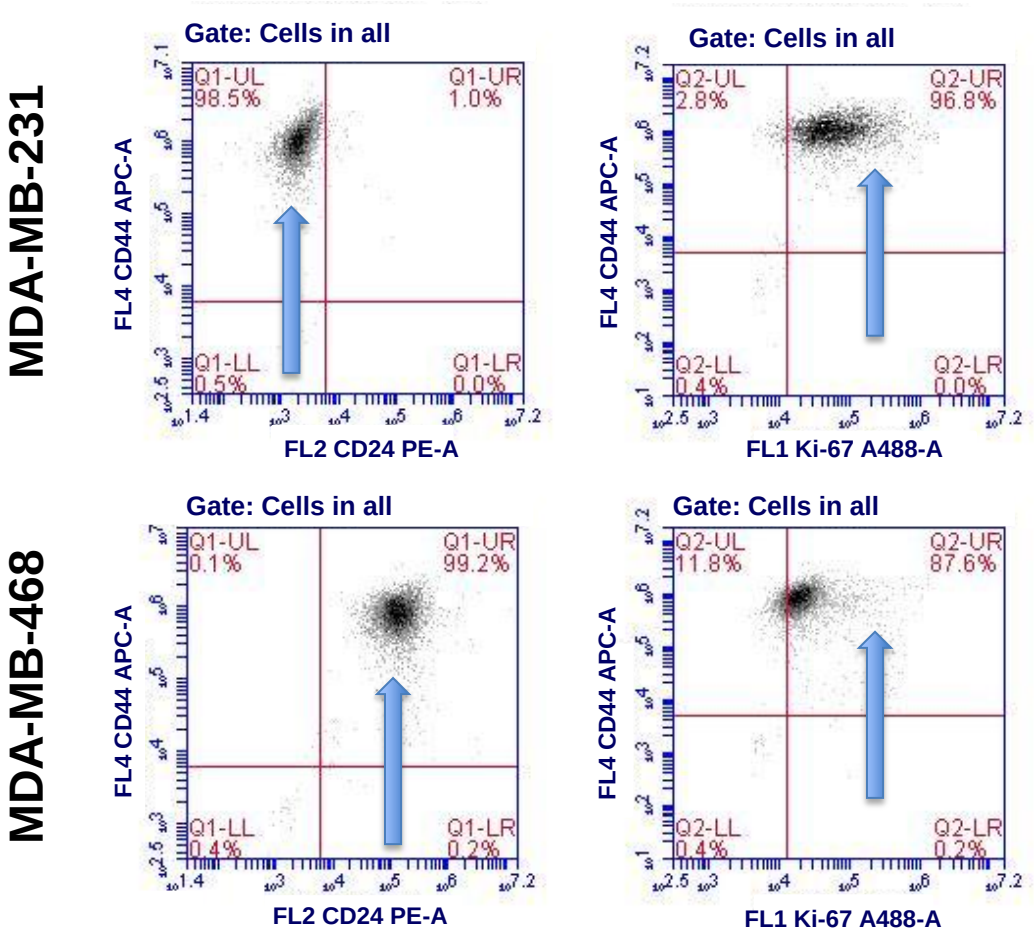
- **Breast cancer cell lines:**
  - **MDA-MB-231**
  - **MDA-MB-468**
  - **MCF-7**
- **Cell function assays:**
  - **Dose-response curve**
  - **Multiparameter functional assays**
- **Immunophenotyping:**
  - **Cell surface and intracellular flow cytometry**
  - **Cell surface marker screening**

Immunophenotyping Breast Cancer Cell Lines

- **CD24 and CD44 surface markers are commonly used to characterize breast cancer cell lines.**

- **$CD44^{+}CD24^{-}$ signature is associated with a more aggressive cancer phenotype:**
  - **Higher proliferation (Ki-67)**
  - **Higher invasion/migration capacity**
  - **Higher expression of soluble factors involved in metastasis (IL-6, IL-8, SDF-1)**

Surface and Intracellular Staining: Ki-67



MDA-MB-231 cells display a cancer stem cell phenotype ($CD44^{+}CD24^{-}$) and express high levels of Ki-67.

Cancer Biology: Cell Surface Marker Screening

January 2013

PLOS ONE

Multiplex Flow Cytometry Barcoding and Antibody Arrays Identify Surface Antigen Profiles of Primary and Metastatic Colon Cancer Cell Lines

Kumar Sukhdeo[1,2*], Rosanto I. Paramban[3], Jason G. Vidal[3], Jeanne Elia[3], Jody Martin[3], Maricruz Rivera[1], Daniel R. Carrasco[4], Awad Jarrar[5,6], Matthew F. Kalady[5,6], Christian T. Carson[3], Robert Balderas[3], Anita B. Hjelmeland[1], Justin D. Lathia[7*], Jeremy N. Rich[1*]

January 2014

Cell Reports
Article

High-Throughput Flow Cytometry Screening Reveals a Role for Junctional Adhesion Molecule A as a Cancer Stem Cell Maintenance Factor

Justin D. Lathia,[1,2,3,4,*] Meizhang Li,[1,2,13] Maksim Sinyuk,[1] Alvaro G. Alvarado,[1,3] William A. Flavahan,[2,3] Kevin Stoltz,[1] Ann Mari Rosager,[5] James Hale,[1] Masahiro Hitomi,[1,3] Joseph Gallagher,[2] Qiulian Wu,[2] Jody Martin,[6] Jason G. Vidal,[6] Ichiro Nakano,[7] Rikke H. Dahlrot,[8] Steinbjørn Hansen,[8] Roger E. McLendon,[9] Andrew E. Sloan,[4,10,11] Shideng Bao,[2,3,4] Anita B. Hjelmeland,[2,3,4] Christian T. Carson,[6] Ulhas P. Naik,[12] Bjarne Kristensen,[5] and Jeremy N. Rich[2,3,4,*]

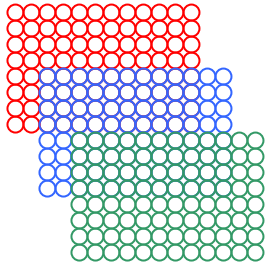
BD Lyoplate™ Screening Panels

Enabling researchers to immunophenotype cell populations by flow cytometry or immunofluorescence microscopy using BD's portfolio of monoclonal antibodies

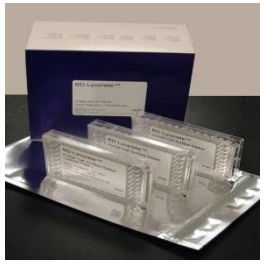
Product	Contents	Size
BD Lyoplate™ *Human* Cell Surface Marker Screening Panel	• 242 CD markers* • Isotype controls • Alexa Fluor®→ 647 second step	5 tests
BD Lyoplate™ *Mouse* Cell Surface Marker Screening Panel	• 176 CD markers • Isotype controls • Biotin second step • Alexa Fluor®→ 647 streptavidin third step	5 tests
*CD and other cell-surface molecules. One marker per well.		

Screening Workflow: Flow Cytometry

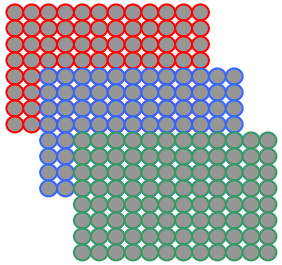
Prepare a single-cell suspension and aliquot into three 96-well plates



Reconstitute BD Lyoplates

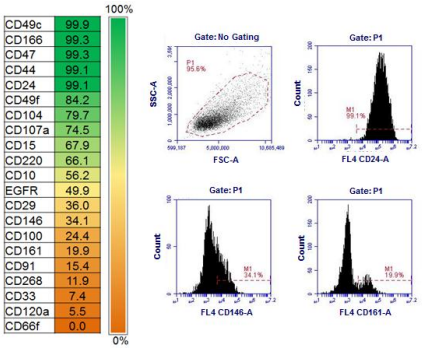


Transfer reconstituted antibodies to plates with cells



Incubate
Wash
Secondary antibody
Wash
Fix Cells
Wash
Collect data

Analyze data

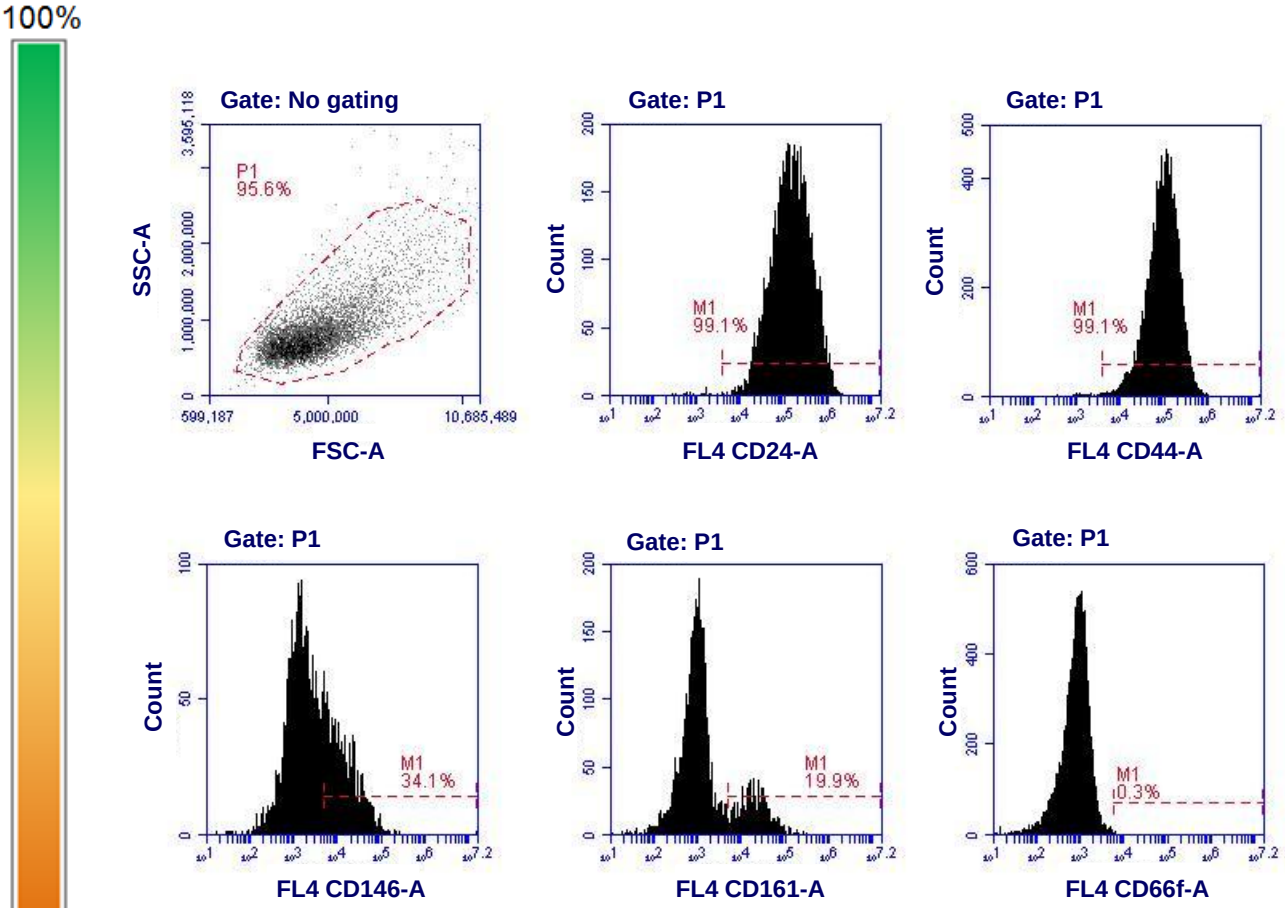


Flow Cytometry within Reach™

The BD Accuri™ C6 Personal Flow Cytometry Tour

Surface Marker Screening of MCF-7 Cells Using the BD Accuri C6

CD49c	99.9
CD166	99.3
CD47	99.3
CD44	99.1
CD24	99.1
CD49f	84.2
CD104	79.7
CD107a	74.5
CD15	67.9
CD220	66.1
CD10	56.2
EGFR	49.9
CD29	36.0
CD146	34.1
CD100	24.4
CD161	19.9
CD91	15.4
CD268	11.9
CD33	7.4
CD120a	5.5
CD66f	0.0



Comparable Screening Results Using BD Accuri C6 or BD FACSCanto™ Flow Cytometer

BD Accuri C6	
CD49c	99.9
CD166	99.3
CD47	99.3
CD44	99.1
CD24	99.1
CD49f	84.2
CD104	79.7
CD107a	74.5
CD15	67.9
CD220	66.1
CD10	56.2
EGFR	49.9
CD29	36.0
CD146	34.1
CD100	24.4
CD161	19.9
CD91	15.4
CD268	11.9
CD33	7.4
CD120a	5.5
CD66f	0.0

BD FACSCanto	
CD49c	99.9
CD166	99.8
CD44	99.6
CD24	99.6
CD47	99.1
CD49f	84.1
CD104	80.3
CD107a	71.4
CD220	69.5
CD15	65.7
CD10	54.9
EGFR	49.6
CD146	41.3
CD29	38.8
CD100	24.0
CD161	23.1
CD91	15.1
CD268	15.0
CD33	9.4
CD120a	6.3
CD66f	0.0

Flow Cytometry Applications in Cell Biology and Cancer Research

- **Cell function assays on flow cytometer**
  - **Apoptosis and viability**
  - **Cell cycle and proliferation**

- **Breast cancer cell lines**
  - **Dose-response curves**
  - **Combination cell function assays**
  - **Cell surface signatures and intracellular flow cytometry**

Acknowledgements

BD Biosciences:

San Diego
- Mirko Corselli
- Lissette Wilensky

Ann Arbor
- David Draper
- Leo Ostruszka
- Stacey Roys

San Jose
- Robert Balderas
- Ranga Partha
- Andy Wang

Flow Cytometry Applications in Cancer Research

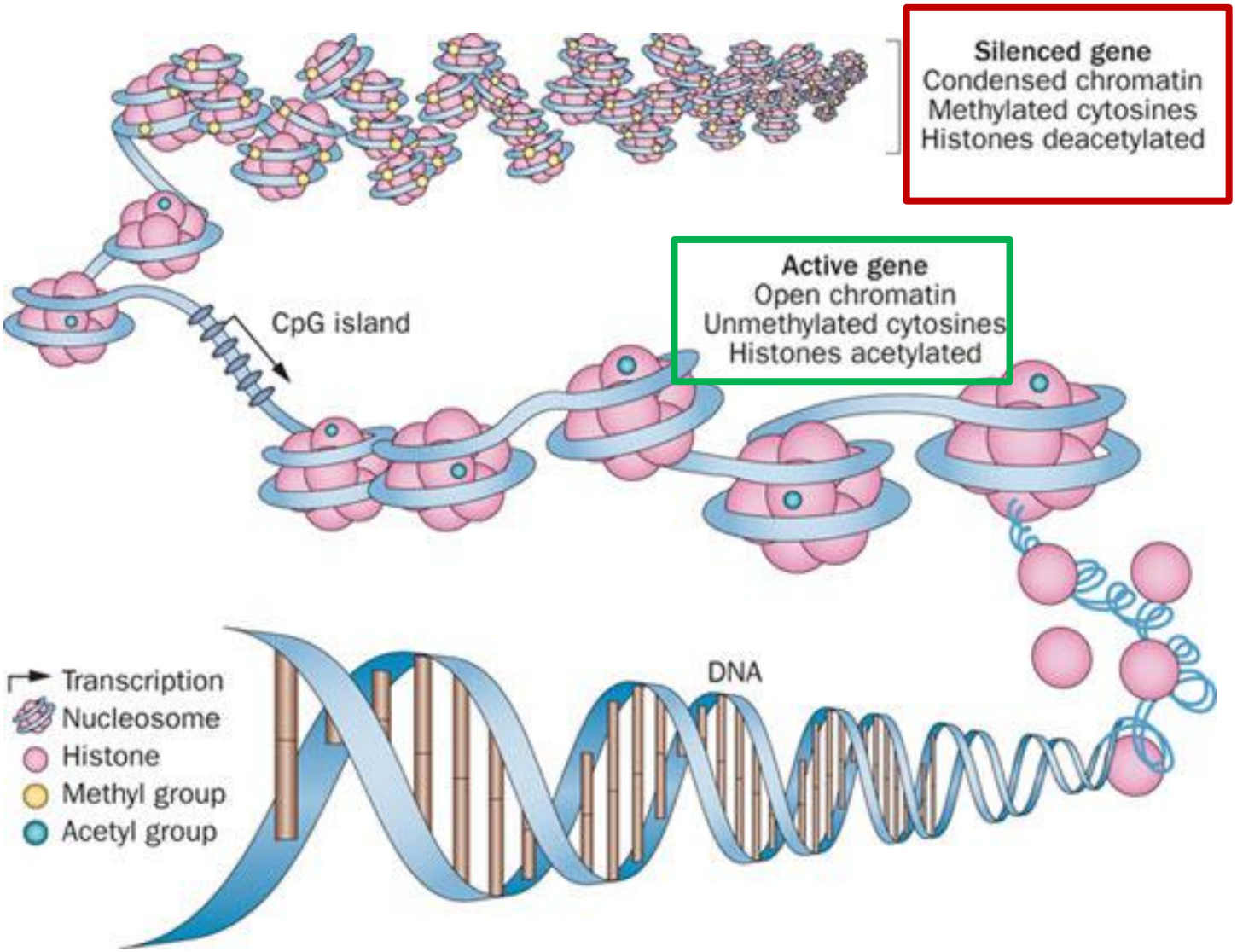
Dr Daniela Trisciuoglio, PhD

Experimental Chemotherapy Laboratory
Regina Elena National Cancer Institute, Rome, Italy

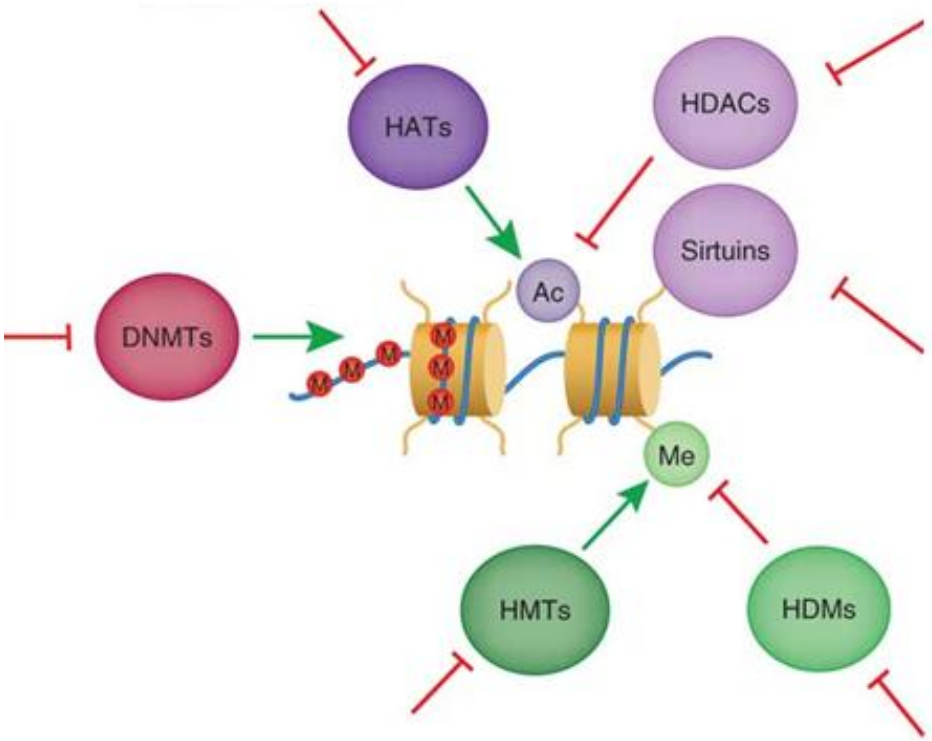
Our Research: Studying new inhibitors of epigenetic enzymes with potential antitumor activity

- ✓ Identify new compounds targeting histone acetyltransferase (HAT) activities and inducing cell death in tumor cells;
- ✓ Study apoptosis induction, cytodifferentiation, and antiproliferative activities induced by histone deacetylase (HDAC) inhibitors in cancer cells;
- ✓ Evaluate the potential antitumoral efficacy of HAT/HDAC inhibitors alone or in combination with antineoplastic drugs;
- ✓ Study the role of bcl-2 family protein in drug-resistance, autophagy and angiogenesis.

Epigenetic regulation of gene expression

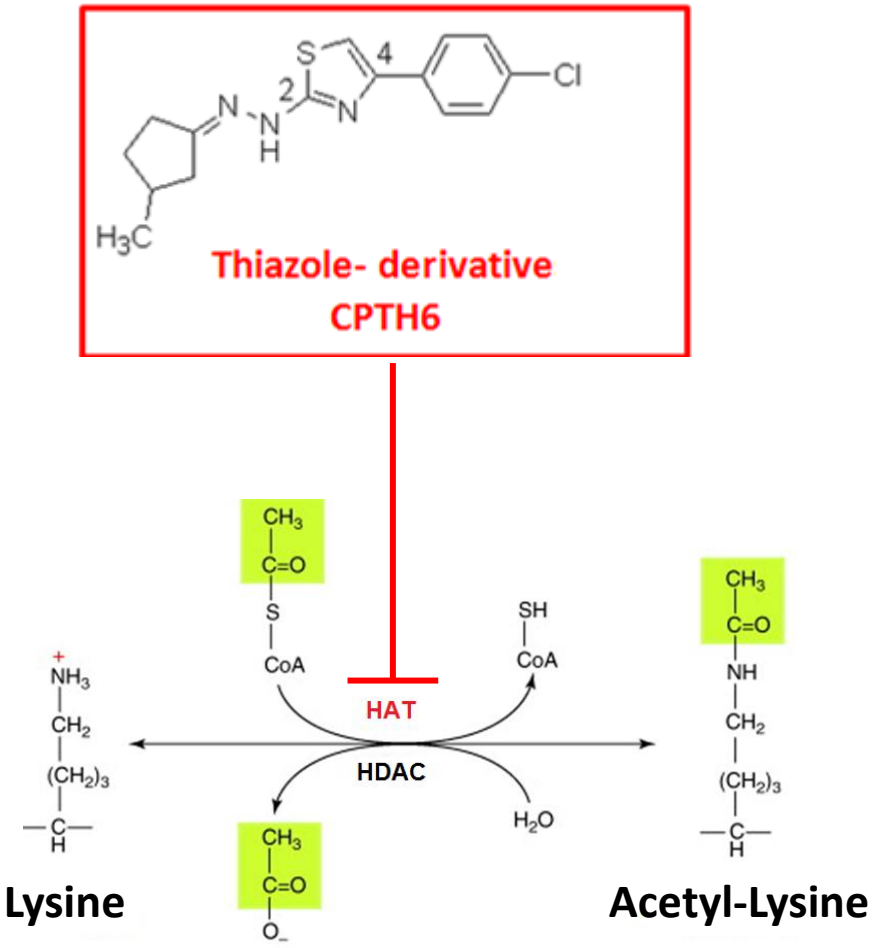


Targeting epigenetic enzymes for cancer therapy



HATs:	histone acetyltransferases
HDACs:	histone deacetylases
HMTs:	histone methyltransferases
HDMs:	histone demethylases
DNMTs:	DNA methyltransferases

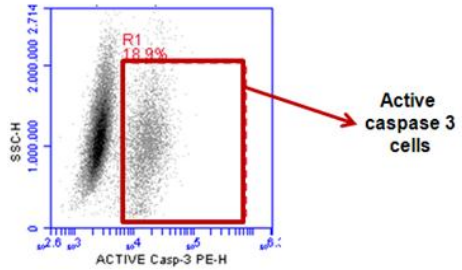
CPTH6, a new histone acetyltransferase inhibitor identified in yeast



- Histone acetyltransferases (HAT): Enzymes influencing transcription mechanisms by selectively acetylating histone proteins.
- HAT components perturbation is typically observed in human tumors.
- HAT are potential targets for antitumor therapy.
- CPTH6 is a new histone acetyltransferase inhibitor identified in yeast.
  Chimenti F, et al .A novel histone acetyltransferase inhibitor modulating Gcn5 network: cyclopentylidene-[4-(4'-chlorophenyl)thiazol-2-yl)hydrazone.
  J Med Chem. 2009 Jan 22;52(2):530-6.

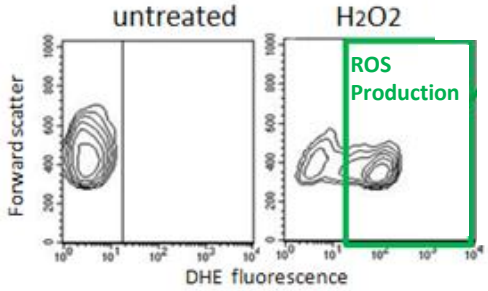
Example of graphics representing flow cytometry results

Dot plot



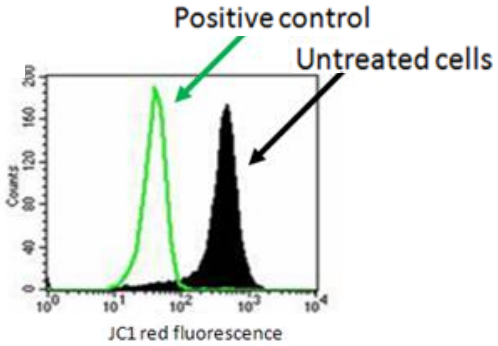
Biparametric analysis to identify cell populations based on fluorescent intensity

Density plot



Biparametric analysis to identify populations of rare cells based on fluorescent intensity

Histogram



Mono parametric analysis to analyze fluorescent intensity level

Focus of the on-going study:
Analysis of the mechanism of action of CPTH6 on acute myeloid leukemia and non-small cell lung cancer cells

Agenda

1. Analysis of CPTH6 specificity as histone acetyltransferase inhibitor and its effect on cancer cells proliferation

2. Analysis of the mechanism of action of CPTH6 on human acute myeloid leukemia cells

3. Analysis of the mechanism of action of CPTH6 on human non-small cell lung cancer cells

4. Conclusion and perspectives

Cancer Therapy: Preclinical

Clinical Cancer Research

CPTH6, a Thiazole Derivative, Induces Histone Hypoacetylation and Apoptosis in Human Leukemia Cells

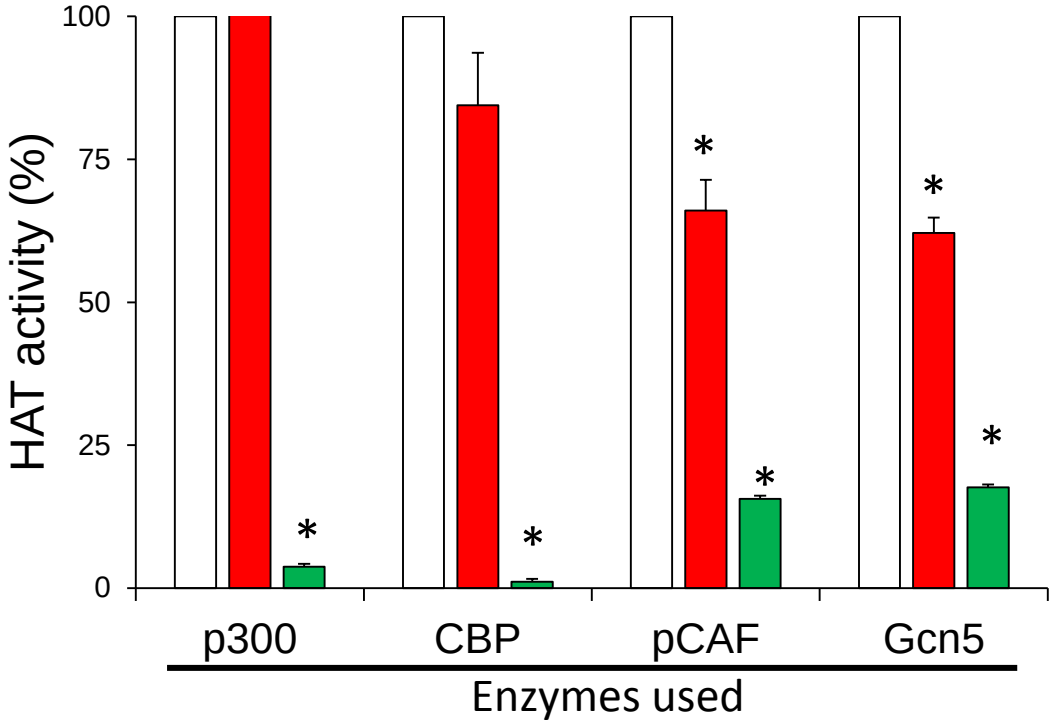
Daniela Trisciuoglio[1], Ylenia Ragazzoni[1], Andrea Pelosi[2], Marianna Desideri[1], Simone Carradori[4], Chiara Gabellini[1,3], Giovanna Maresca[7], Riccardo Nescatelli[5], Daniela Secci[4], Adriana Bolasco[4], Bruna Bizzarri[4], Chiara Cavaliere[5], Igea D'Agnano[7], Patrizia Filetici[6], Lucia Ricci-Vitiani[8], Maria Giulia Rizzo[2], and Donatella Del Bufalo[1]

Clin Cancer Res. 2012 Jan 15;18(2):475-86

Contact details:

- Dr. Daniela Trisciuoglio - trisciuoglio@ifo.it;
- Dr. Daniela Del Bufalo - delbufalo@ifo.it

CPTH6 is a specific Gcn5/pCAF inhibitor



HAT activity assay: In vitro assay using purified recombinant enzymes with H3 histone and [^{3}H]acetyl-CoA

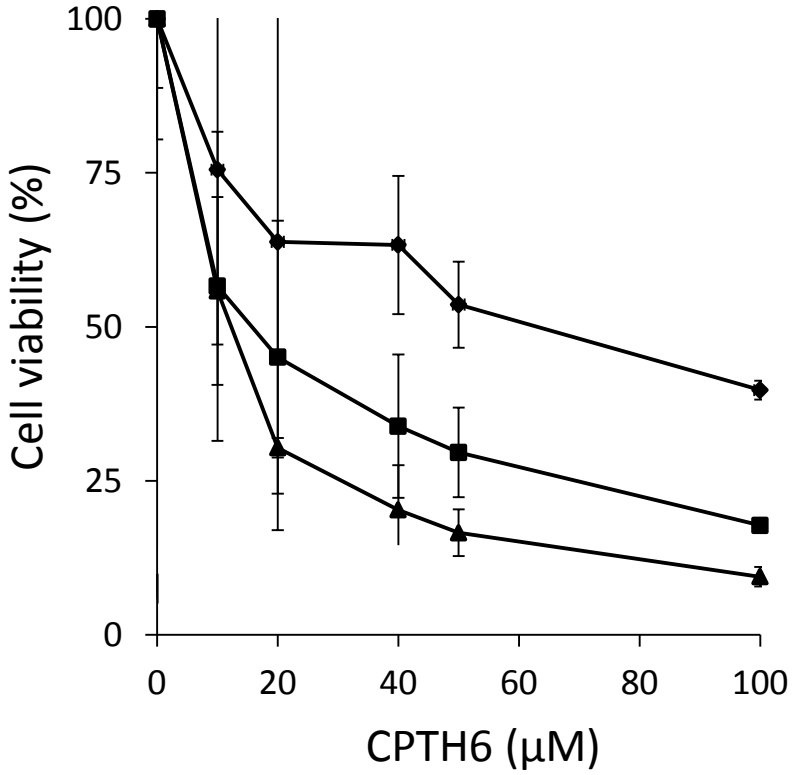
□ Vehicle

■ CPTH6

■ AA (Anacardic acid - panHAT inhibitor)

Note: Western blot analyses showed a significant reduction of H3 histone, H4 histone and α-tubulin acetylation in a time- and concentration-dependent manner (Results not shown).

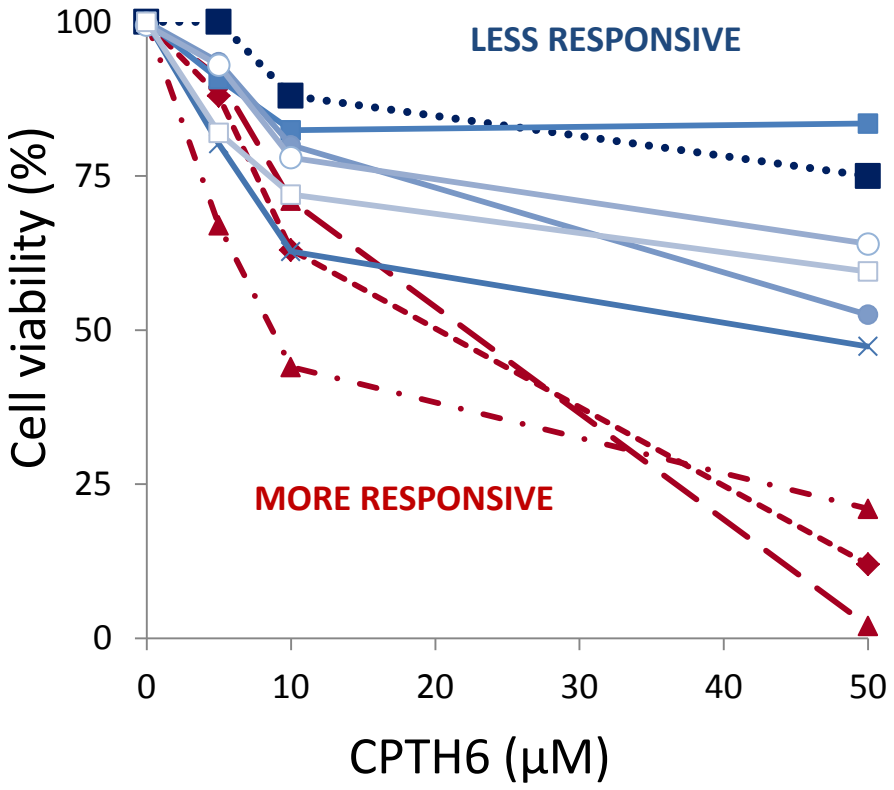
CPTH6 reduces in vitro cell viability of U937 acute myeloid leukemia (AML) cells



MTT Cell viability assay:
Colorimetric assay based on the reduction of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] tetrazolium.

◆ 24h
■ 48h
▲ 72h

CPTH6 preferentially decrease cell viability of non-small cell lung cancer (NSCLC) patient-derived stem-like (LCSC) cells in vitro



Established commercial cell lines,
Colorimetric assay based on the reduction of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] tetrazolium.

- H1299
- H460
- Calu-1
- Calu-3
- A549

Patient-derived stem-like cells,
Luminescent Cell Viability Assay based on quantitation of the ATP present.

- LCSC 136
- LCSC 36
- LCSC 34
- LCSC 143

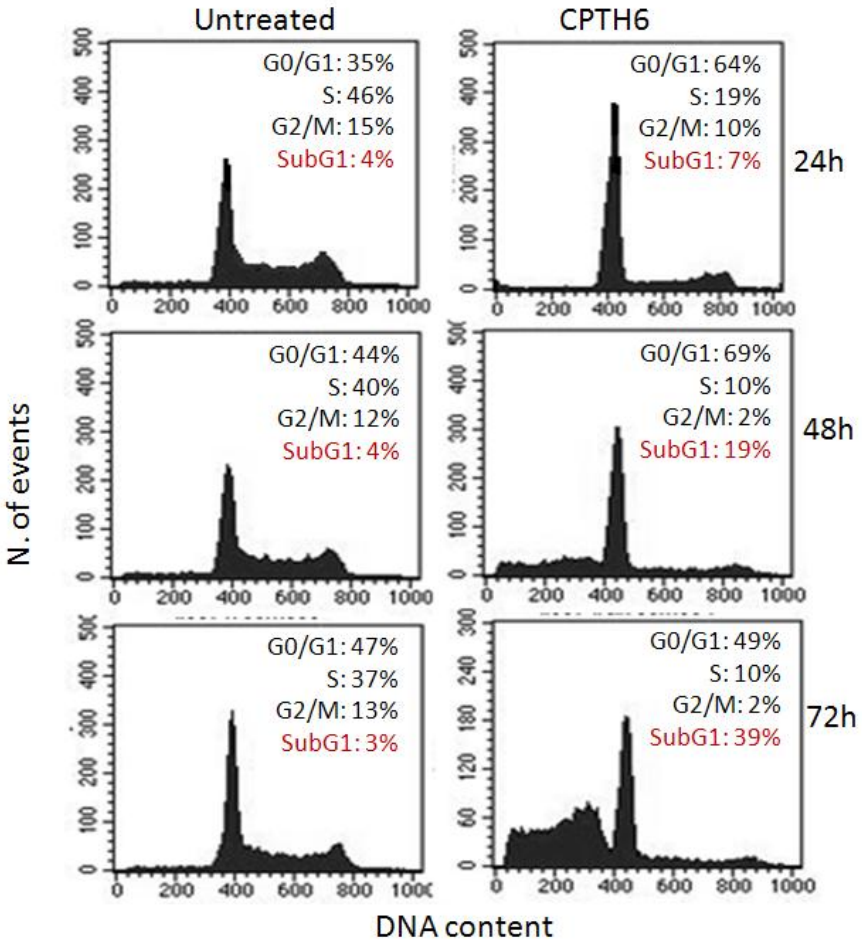
Summary 1

- ✓ CPTH6 is a specific Gcn5/pCAF inhibitor and reduces significantly α-tubulin, histone H3 and H4 acetylation in a time- and concentration-dependent manner in human acute myeloid leukemia cells

- ✓ CPTH6 reduces *in vitro* cell viability of human acute myeloid leukemia cells

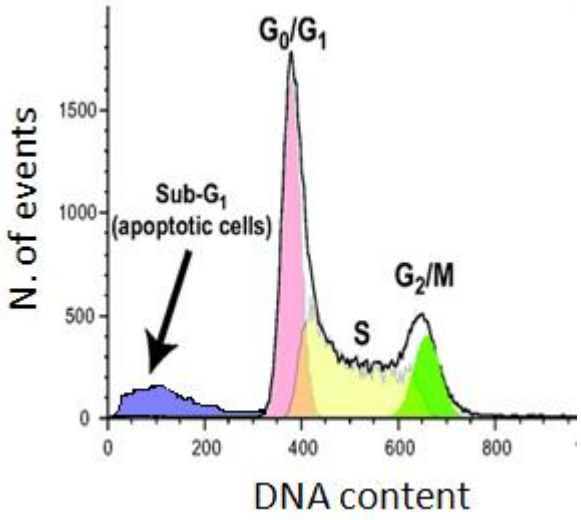
- ✓ CPTH6 differently affects *in vitro* cell viability of human non- small cell lung cancer cell lines

CPTH6 induces cell-cycle perturbation in U937 human acute myeloid leukemia (AML) cells

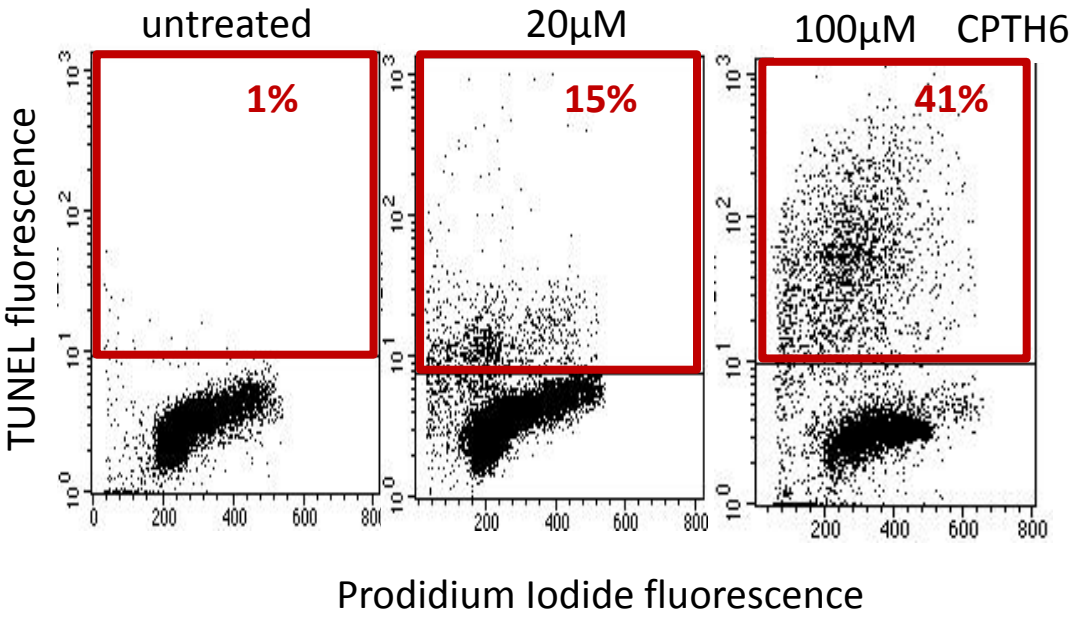


Flow cytometric analysis of DNA content: Determination of fluorescent intensity of propidium iodide intercalated in DNA.

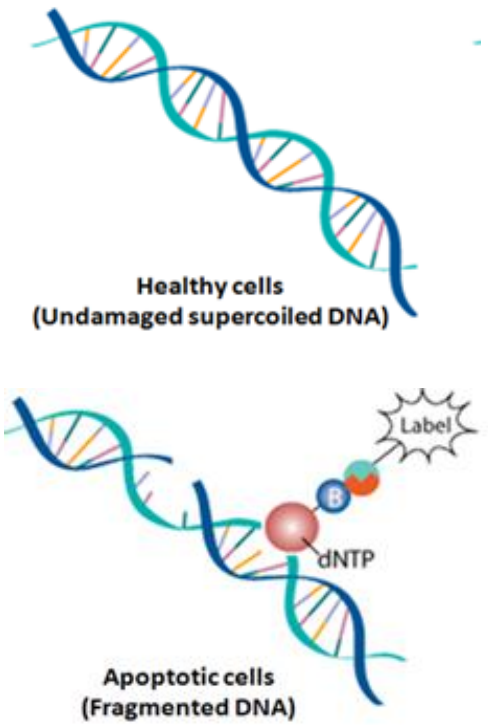
Example of profile:



CPTH6 induces apoptosis in U937 AML cells

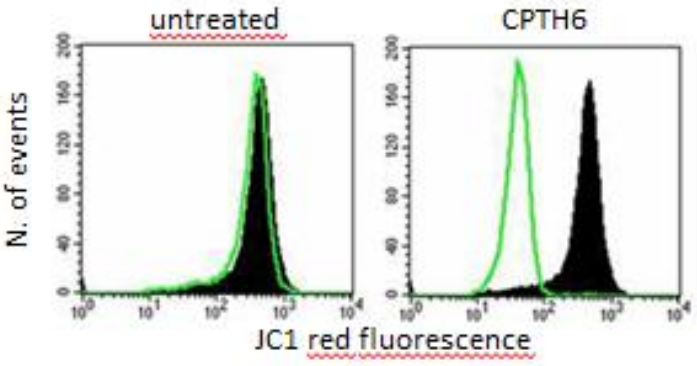


Flow cytometric TUNEL assay:
Apoptosis detection by a two-color assay for labeling DNA breaks and total cellular DNA content.

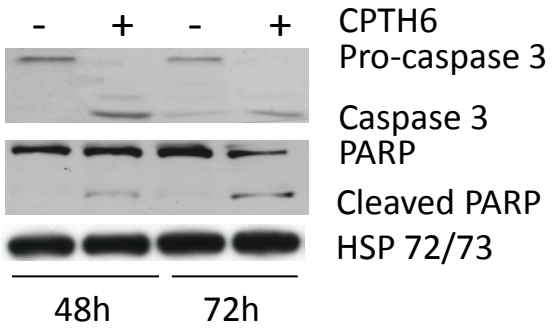


Determination of apoptotic mechanisms induced by CPTH6 treatment in U937 cells

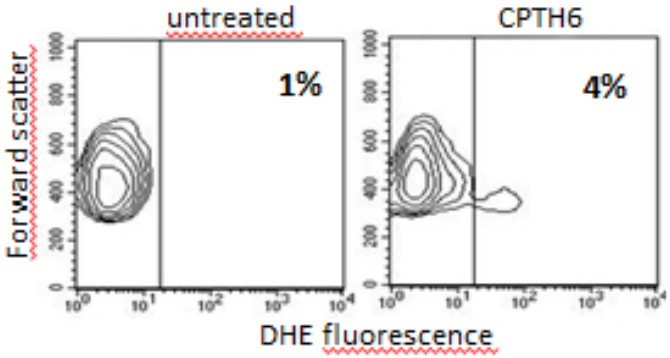
Mitochondrial membrane potential flow cytometric assay (JC1 probe)



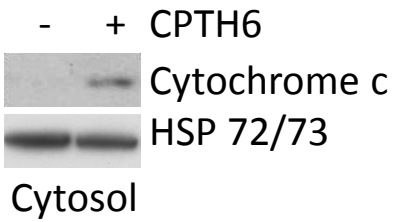
Western Blot analysis:
Detection of apoptotic markers



Flow cytometric assay detecting Reactive Oxygen Species production (DHE)



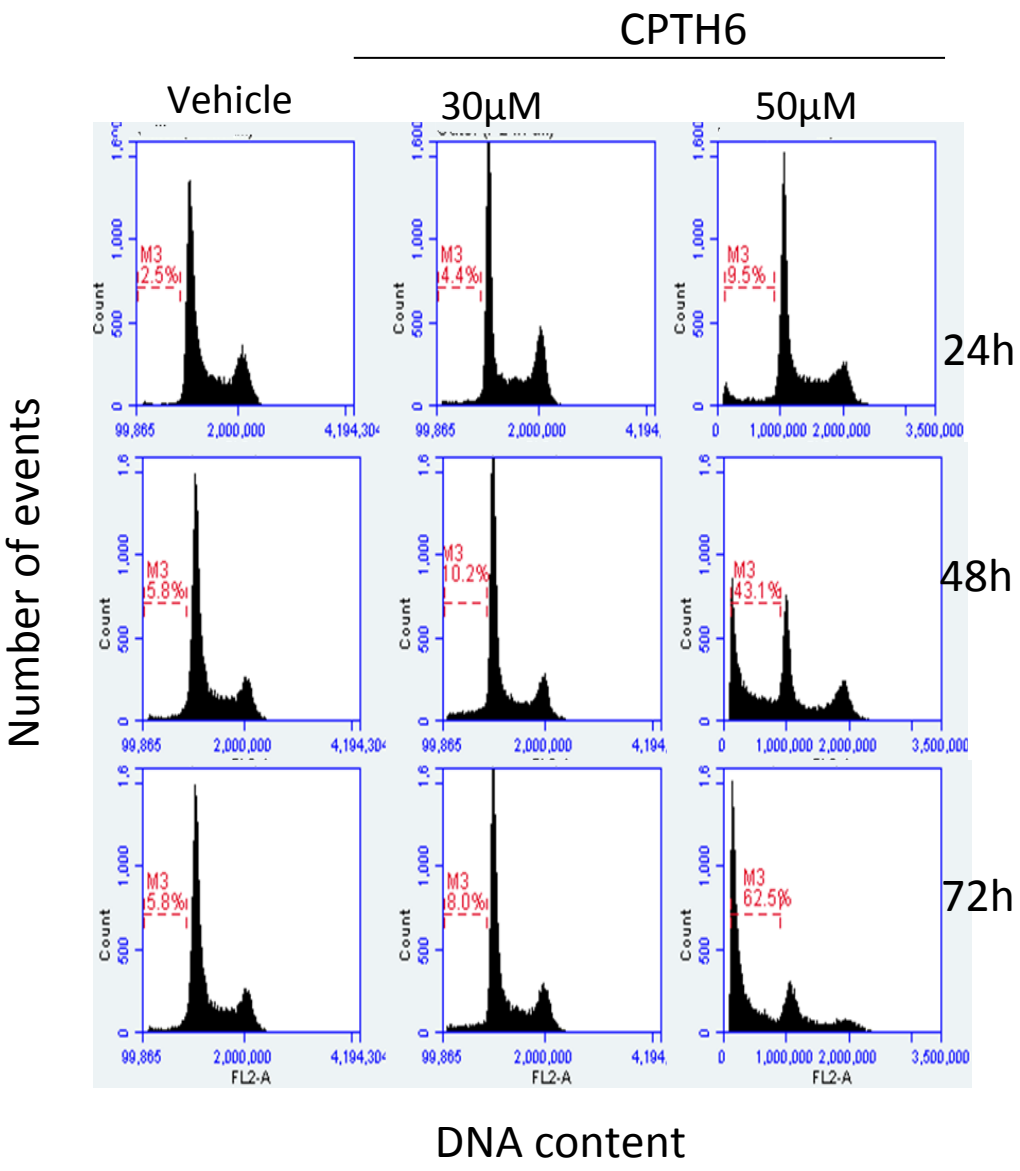
Extraction and Western Blot analysis:
Detection of an apoptotic marker in cytosol



Summary 2

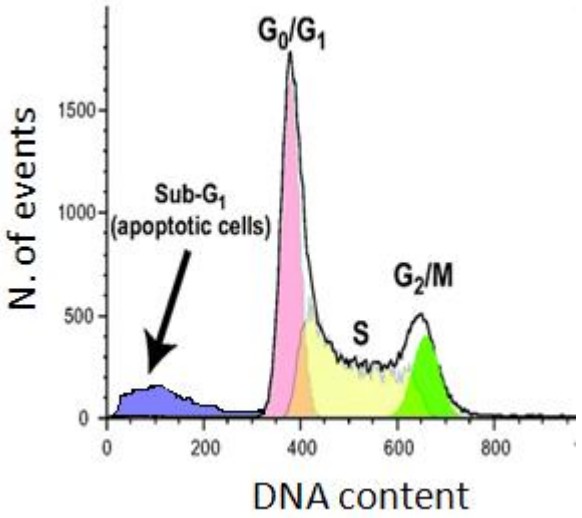
- ✓ CPTH6 is a specific Gcn5/pCAF inhibitor and reduces significantly α-tubulin, histone H3 and H4 acetylation in a time- and concentration-dependent manner in human acute myeloid leukemia cells
- ✓ CPTH6 reduces *in vitro* cell viability of human acute myeloid leukemia cells
- ✓ CPTH6 differently affects *in vitro* cell viability of human non- small cell lung cancer cell lines
- ✓ CPTH6 induces cell-cycle perturbation and apoptosis in U937 AML cells
- ✓ CPTH6 induces intrinsic apoptotic pathway activation in U937 AML cells

CPTH6 induces apoptosis in patient-derived stem-like LCSC136 cells

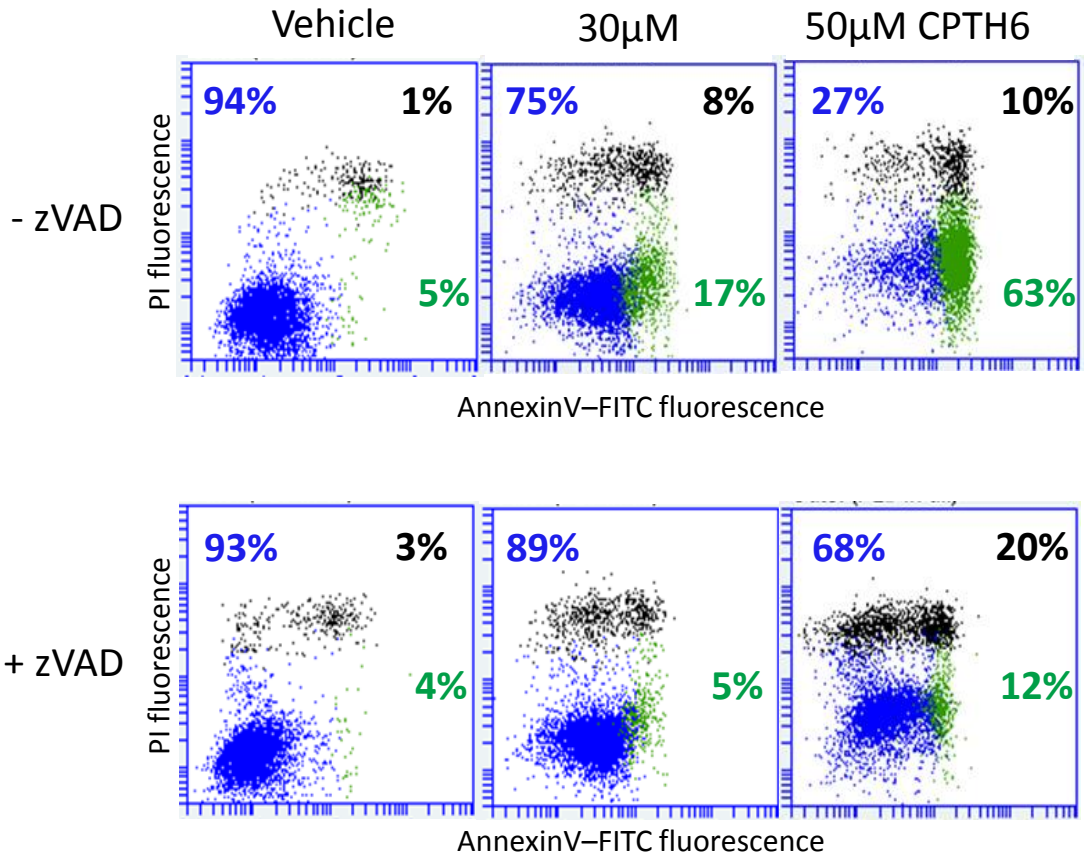


Flow cytometric analysis of DNA content: Determination of fluorescent intensity of propidium iodide intercalated in DNA.

Example of profile:

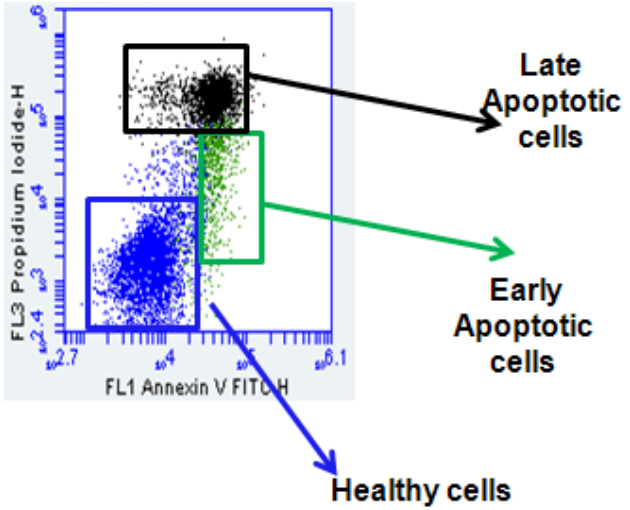


CPTH6 induces specific alterations to the plasma membrane, hallmark of apoptotic cells, in LCSC136 patient-derived lung cancer stem cell line.



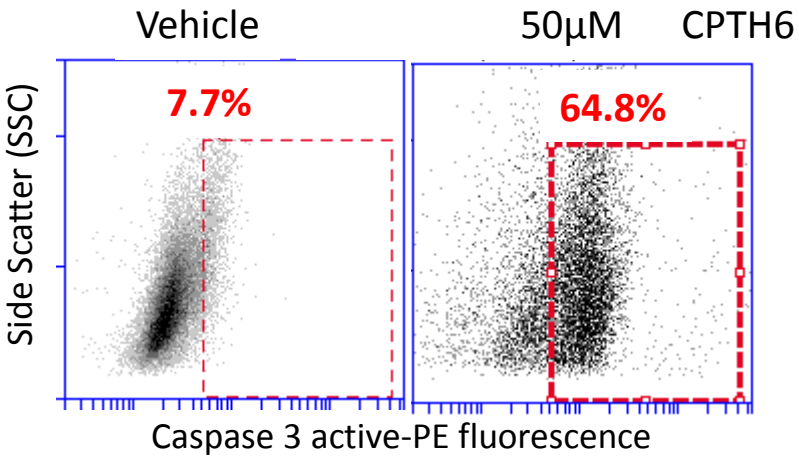
Flow cytometric AnnexinV binding assay:
Fluorescent Annexin V binding to altered plasma membrane of apoptotic cells.

Example of profile:



Note: zVAD is a pan caspase inhibitor

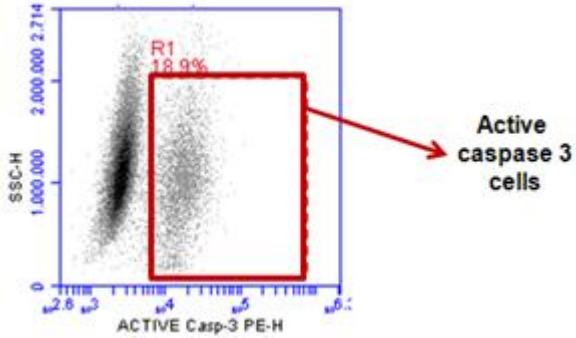
CPTH6 induces caspase 3 activation and cleavage of PARP in LCSC136 patient-derived lung cancer stem cell line

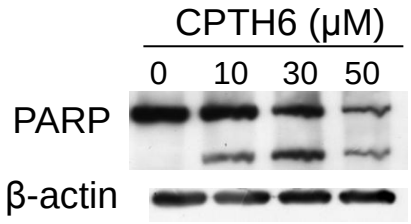


Flow cytometric active caspase 3 detection assay

Detection of the caspase 3 active form using an anti-active caspase-3 antibody.

Example of profile

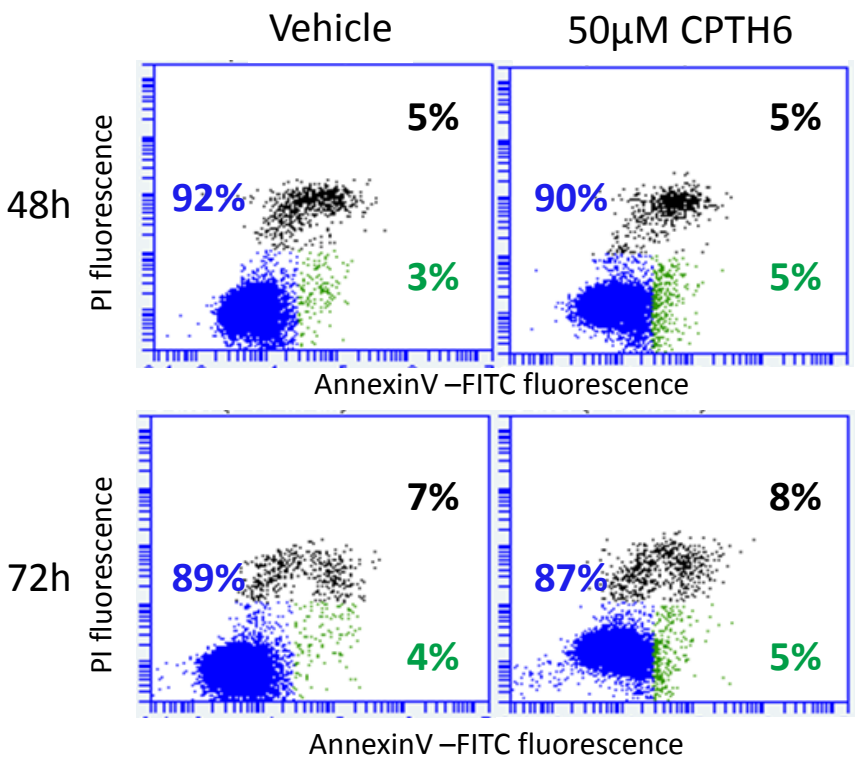




Western Blot analysis:

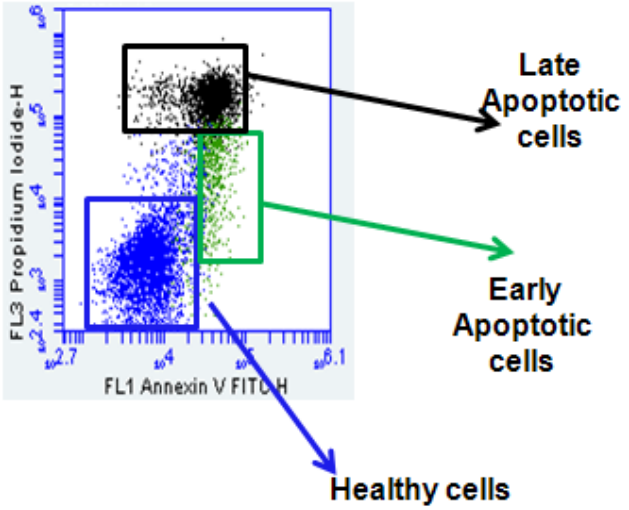
Detection of cleaved PARP protein, an apoptotic marker

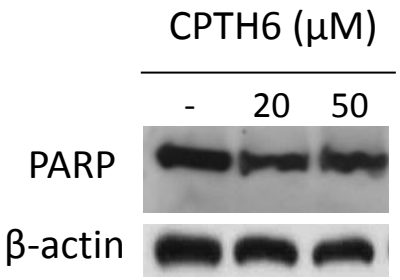
CPTH6 does NOT induce apoptosis in established human H1299 NSCLC cell line



Flow cytometric AnnexinV binding assay:
Fluorescent Annexin V binding to altered plasma membrane of apoptotic cells.

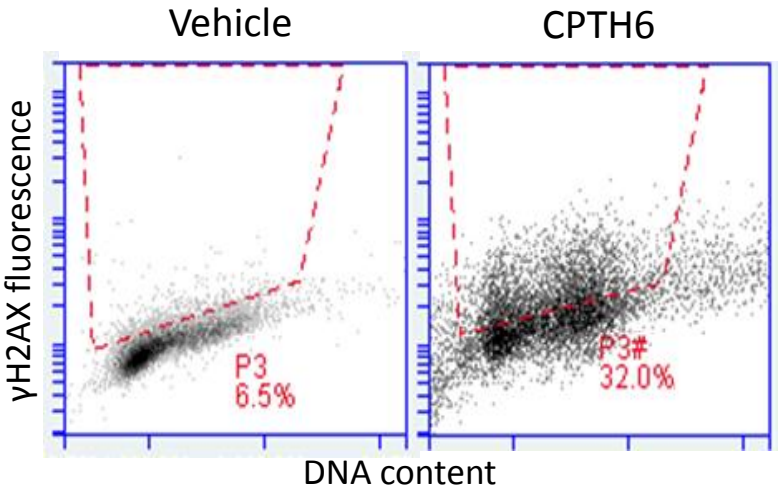
Example of profile:



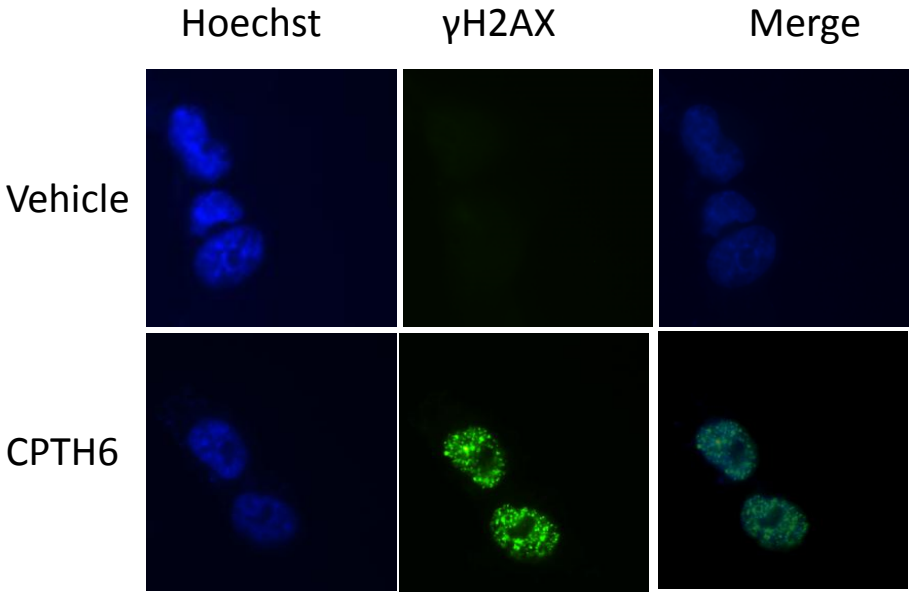


Western Blot analysis:
Detection of cleaved PARP, an apoptotic market

CPTH6 induces DNA damage in established human H1299 NSCLC cell line



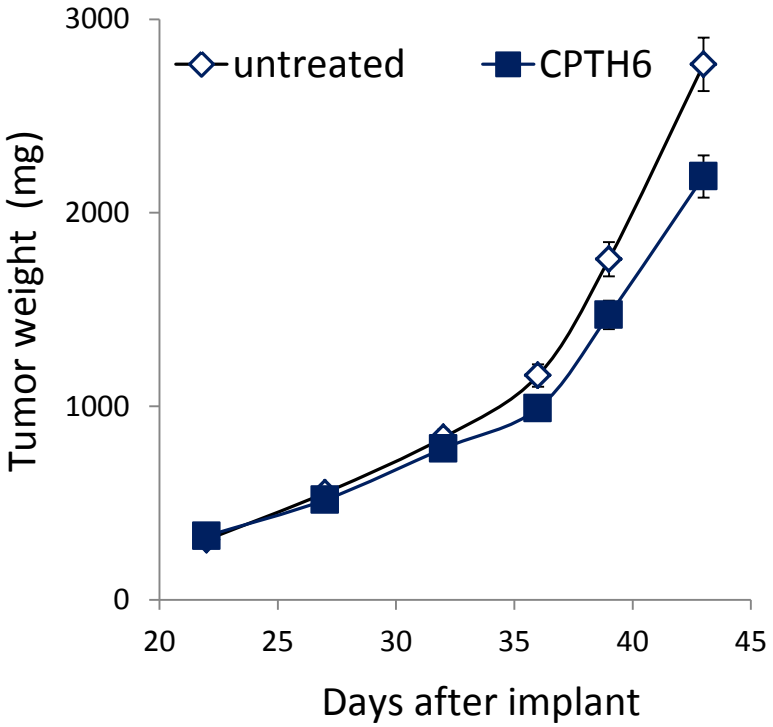
Flow cytometric γH2AX assay
Detection of H2AX phosporylation, a marker for DNA damage, using a H2AX antibody.



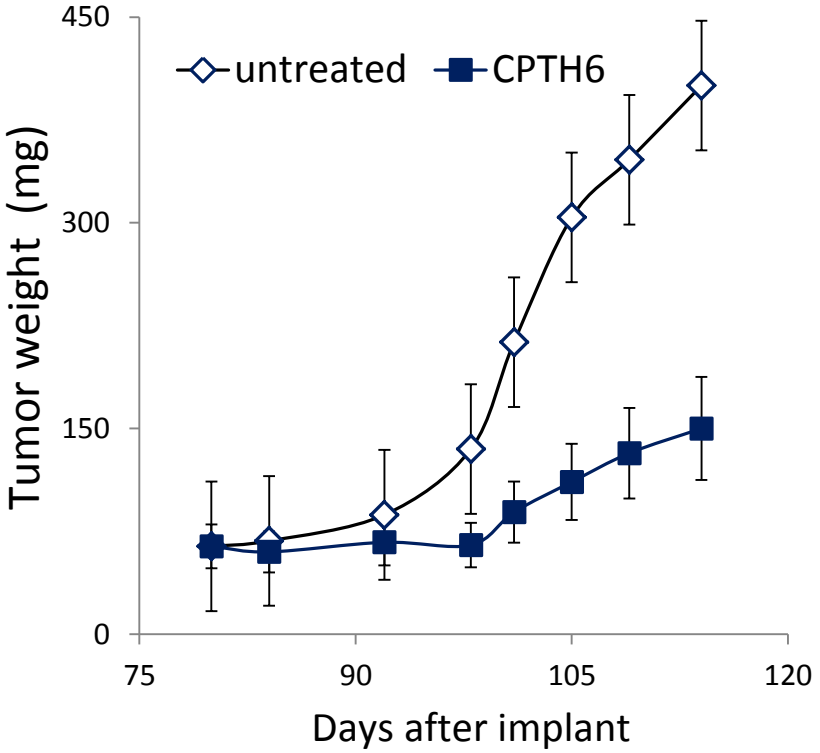
Immunofluorescence analysis of H2AX phosporylation

CPTH6 preferentially inhibits in vivo growth of NSCLC patient-derived cancer stem xenografts

H1299
(established human NSCLC cell line)



LCSC136
(patient-derived lung cancer stem cell line)



CPTH6, 50 mg/Kg/day for 3 weeks

Summary 3

- ✓ CPTH6 is a specific Gcn5/pCAF inhibitor and reduces significantly α-tubulin, histone H3 and H4 acetylation in a time- and concentration-dependent manner in human acute myeloid leukemia cells
- ✓ CPTH6 reduces *in vitro* cell viability of human acute myeloid leukemia cells
- ✓ CPTH6 differently affects *in vitro* cell viability of human non- small cell lung cancer cell lines
- ✓ CPTH6 induces cell-cycle perturbation and apoptosis in U937 AML cells
- ✓ CPTH6 induces intrinsic pathway activation in U937 AML cells
- ✓ CPTH6 induces apoptosis in patient-derived stem-like LCSC136 cells
- ✓ CPTH6 induces DNA damage in human NSCLC cell lines but does not activate apoptosis
- ✓ CPTH6 preferentially inhibits in vivo growth of NSCLC patient-derived cancer stem xenograft

Conclusions and perspectives

These results make CPTH6 a suitable tool for discovery of molecular targets of HAT and, potentially, for the development of new anticancer therapies, which warrants further investigations.

1. To better define the molecular targets of CPTH6 action by proteomic and gene expression studies.

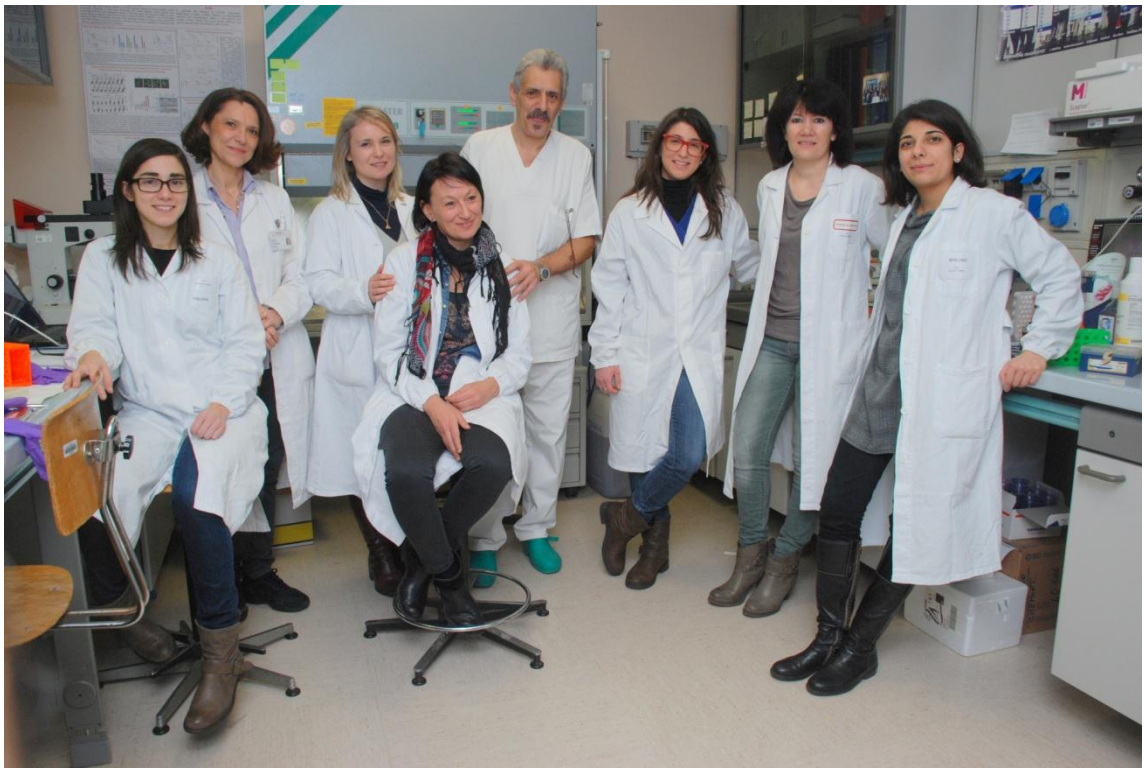
2. To evaluate the potential antitumoral efficacy of CPTH6 alone or in combination with cytotoxic agents currently used in NSCLC therapy.

Acknowledgements

Regina Elena National Cancer Institute, Rome
Marta DI MARTILE
Marianna DESIDERI
Teresa DE LUCA
Chiara GABELLINI
Simona D'AGUANNO
Valentina FARINI
Michele MILELLA
Ruggero DE MARIA

Donatella DEL BUFALO

Istituto Superiore di Sanità, Rome
Adriana ERAMO
Sapienza University, Rome
Daniela SECCI
Simone CARRADORI



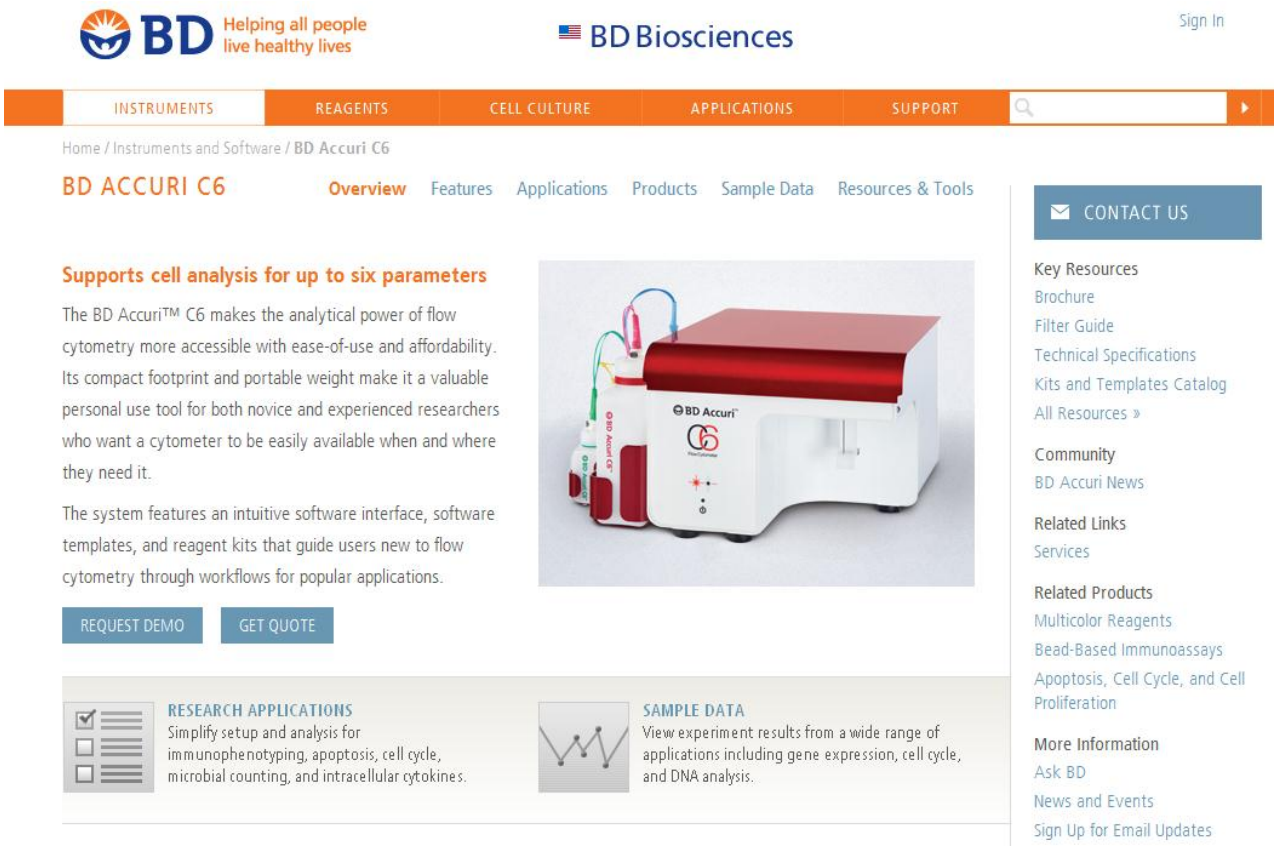
AIRC Con la ricerca, contro il cancro.

Fondazione Umberto Veronesi
PER IL PROGRESSO DELLE SCIENZE

Resources: Kits and Templates

Category	Product Information Sheet	Brand	Kit	Cat. No.	Template
Cell Biology	BD Apoptosis Kits and Templates	BD Pharmingen™	Annexin V FITC Apoptosis Detection Kit II	556570	Download
		BD Pharmingen™	Annexin V PE Apoptosis Detection Kit I	559763	Download
		BD™	MitoScreen (JC-1) Kit	551302	Download
		BD Pharmingen™	Caspase-3 PE Assay Kit	550914	Download
		BD Pharmingen™	Caspase-3 FITC Assay Kit	550480	Download
	BD Apoptosis, DNA Damage and Cell Proliferation Kit and Template	BD Pharmingen™	Apoptosis, DNA Damage and Cell Proliferation Kit	562253	Download
	BD Cell Cycle and DNA Kits and Templates	BD Cycletest™ Plus	DNA Reagent Kit	340242	Download
		BD Pharmingen™	FITC BrdU Flow Kit	559619	Download
		BD Pharmingen™	APC BrdU Flow Kit	552598	Download

Additional Resources



www.bdbiosciences.com/resources/accuri

Technical Support:

Ph: 877-232-8995, Prompt 3, 2

email: ResearchApplications@bd.com

An Unprecedented Special Offer (US Only)

Great savings on the BD Accuri™ C6 system

Flow Cytometry Even More Within Reach™

$7,000 *off* the BD Accuri™ C6

or

$12,000 *off* the BD Accuri™ C6 and BD CSampler™

www.bdbiosciences.com/go/accuri

www.bdbiosciences.com/go/cancerbiology/

Questions?