The Modified Phenanthridine PJ34 Unveils a Cell-Death Mechanism Exclusive to Human Cancer Cells

The post translational modification of NuMA is a new target for cancer therapy

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Faculty of Medicine and Sagol School of Neuroscience
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Tricyclic molecules eradicating human cancer cells

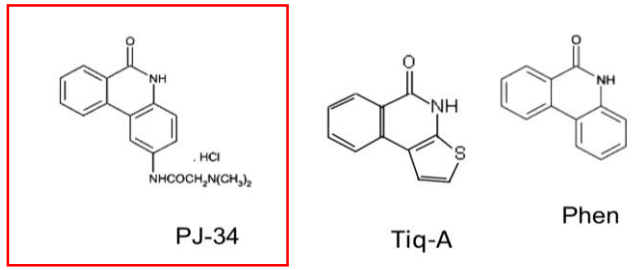
Castiel *et al. BMC Cancer* 2011, **11**:412
http://www.biomedcentral.com/1471-2407/11/412

BMC Cancer

RESEARCH ARTICLE Open Access

A phenanthrene derived PARP inhibitor is an extra-centrosomes de-clustering agent exclusively eradicating human cancer cells

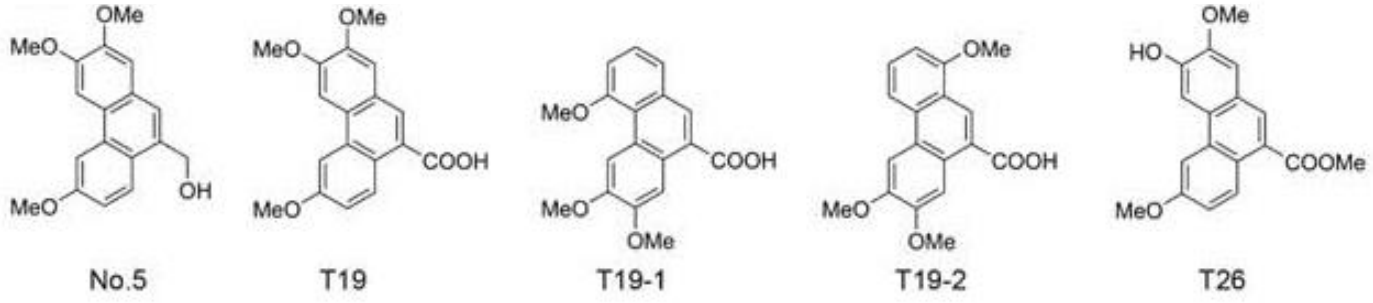
Asher Castiel[4†], Leonid Visochek[1†], Leonid Mittelman[3], Françoise Dantzer[5], Shai Izraeli[2,4] and Malka Cohen-Armon[1*]



Cancer Science
The official journal of the Japanese Cancer Association
JCA

Identification of a phenanthrene derivative as a potent anticancer drug with Pim kinase inhibitory activity

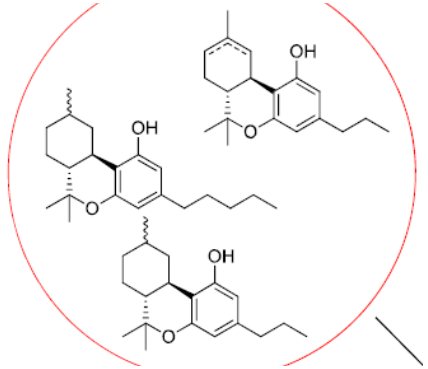
Ying-Ying Wang,[1] Tsuyoshi Taniguchi,[2] Tomohisa Baba,[1] Ying-Yi Li,[1,3,4] Hiroyuki Ishibashi[2] and Naofumi Mukaida[1,5]



Antineoplastic Properties of THCV, HHC and their anti-Proliferative effects on HPAF-II, MIA-paca2, Aspc-1, and PANC-1 PDAC Pancreatic Cell Lines

Tesfay T. Tesfatsion[1], Arianna C. Collins[1], Giovanni A. Ramirez[1], Yousef Mzannar[2], Husain Yar Khan[2], Omar Aboukameel[2], Asfar S. Azmi[2], Prakash G. Jagtap[1], Kyle P. Ray[1,3], Westley Cruces[1,3]

[1] *Colorado Chromatography Labs LLC., 10505 S Progress Way Unit 105 Parker CO 80134*
[2] *Karmanos Cancer Institute, Wayne State University, 4100 John R. St, Detroit, MI 48201*
[3] *BlackStone Therapeutics, 10505 S Progress Way Unit 105 Parker CO 80134*



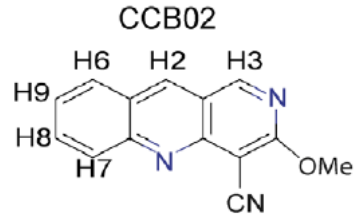
Article

TRANSPARENT PROCESS OPEN ACCESS

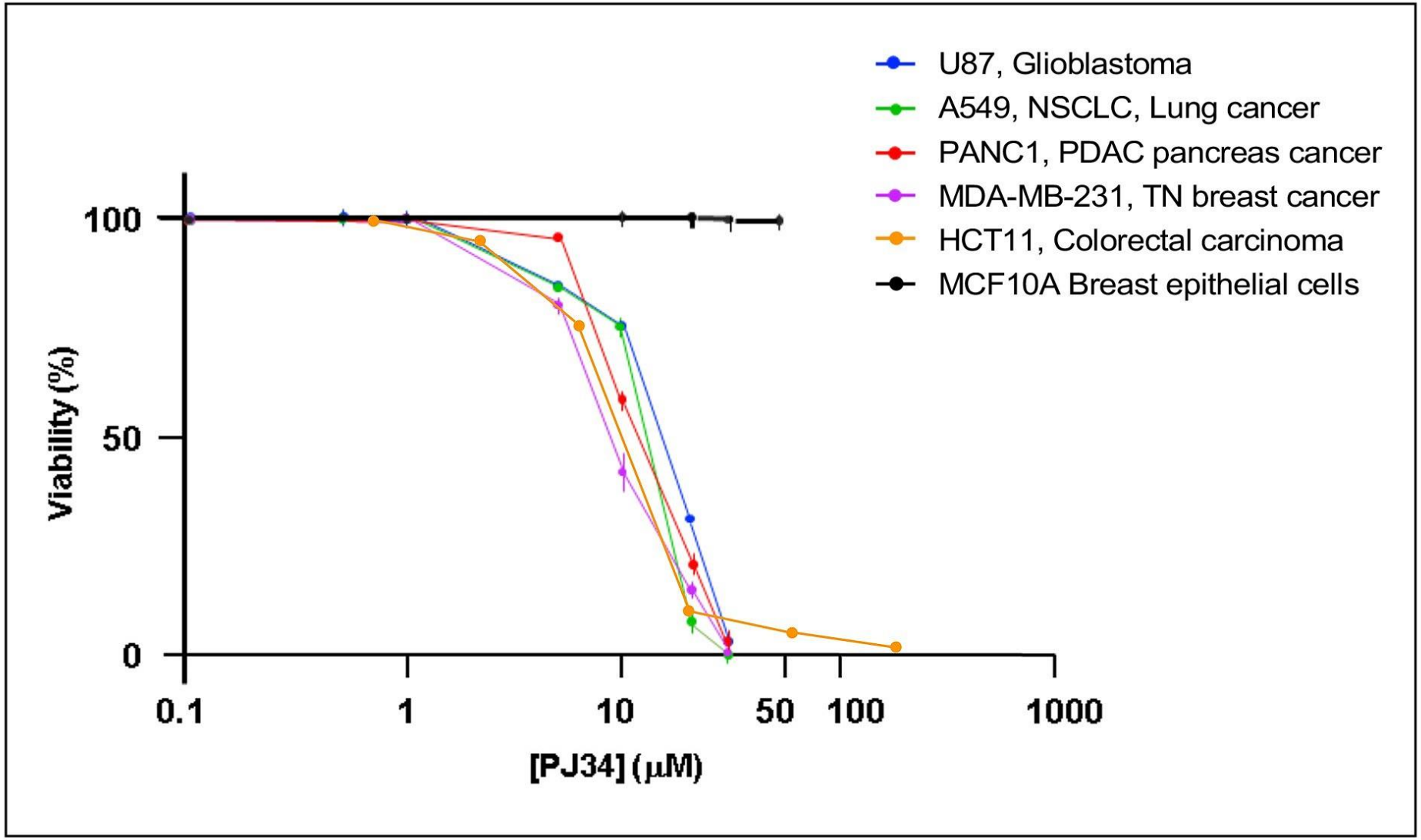
THE EMBO JOURNAL

Inhibition of CPAP–tubulin interaction prevents proliferation of centrosome-amplified cancer cells

Aruljothi Mariappan[1,2], Komal Soni[3,4], Kenji Schorpp[5], Fan Zhao[6,7,8], Amin Minakar[9], Xiangdong Zheng[6,7,8], Sunit Mandad[10,11,12], Iris Macheleidt[13], Anand Ramani[1,14], Tomáš Kubelka[4], Maciej Dawidowski[3,4,15], Kristina Golfmann[2], Arpit Wason[2], Chunhua Yang[16], Judith Simons[2], Hans-Günther Schmalz[9], Anthony A Hyman[17], Ritu Aneja[16], Roland Ullrich[2], Henning Urlaub[10,11], Margarete Odenthal[13], Reinhardt Büttner[13], Haitao Li[6,7,8], Michael Sattler[3,4], Kamyar Hadian[5] & Jay Gopalakrishnan[1,2,14,*]



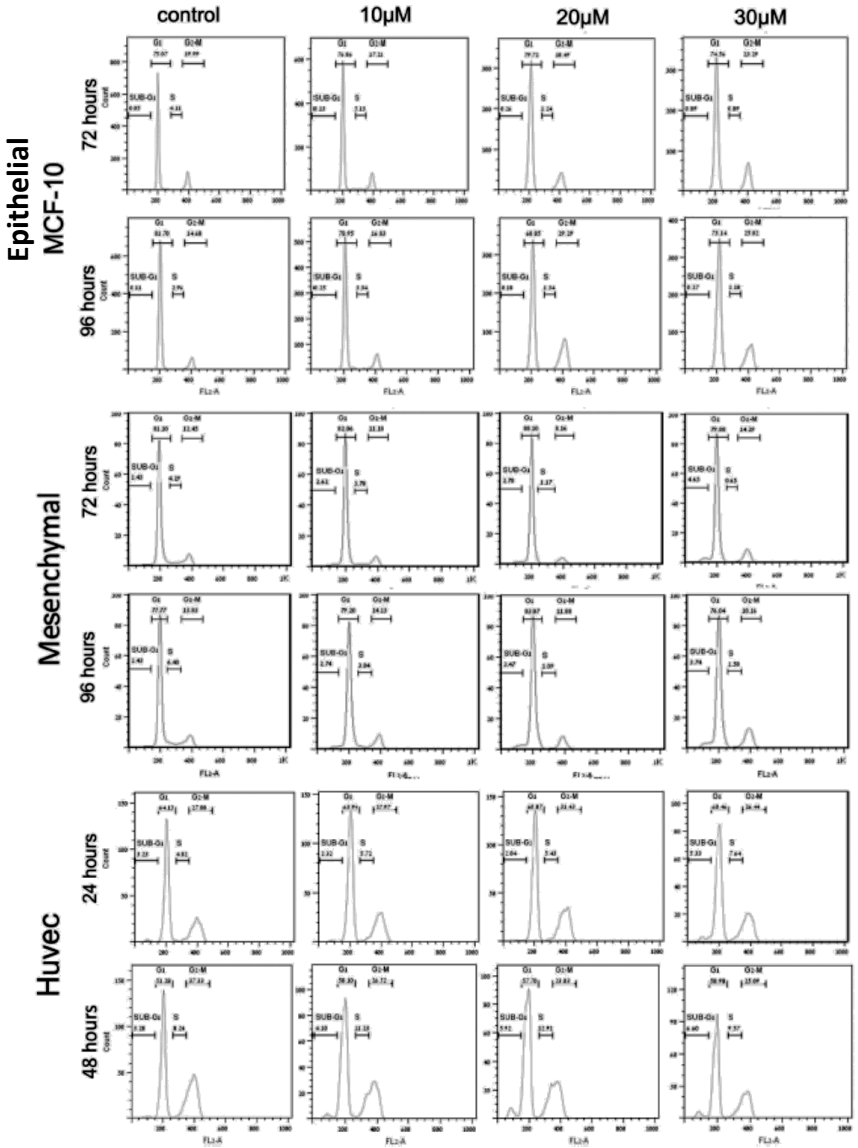
Exclusive eradication of the indicated human malignant epithelial cells treated with PJ34 (96 hours) at the indicated concentrations. Benign human breast epithelial cells are not impaired.

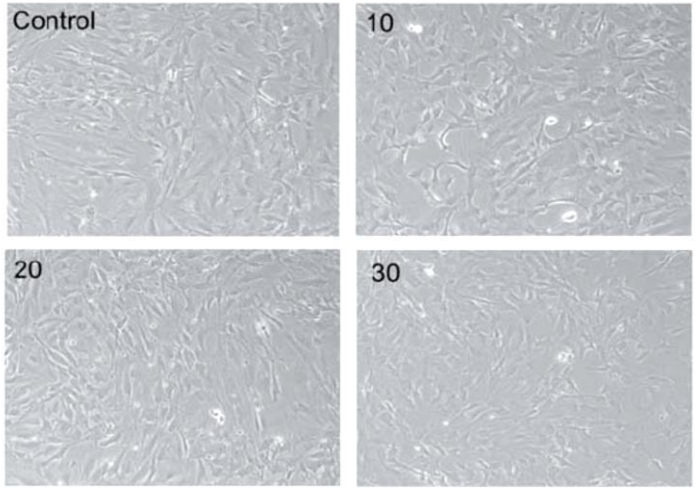


Cohen-Armon, Cell Death & Disease, 2025

PJ34 Does Not impair the Cell Cycle of proliferating healthy somatic cells

PJ34 does not affect the cell-cycle in human healthy somatic cells (breast epithelial cells, primary human thymus mesenchymal cells and human endothelial cells (prepared from the Human Umbilical vein) treated with PJ34 in the indicated concentration and incubation periods





Proliferation of human breast epithelial cells incubated with PJ34 at the indicated concentrations (μM) for 96 h

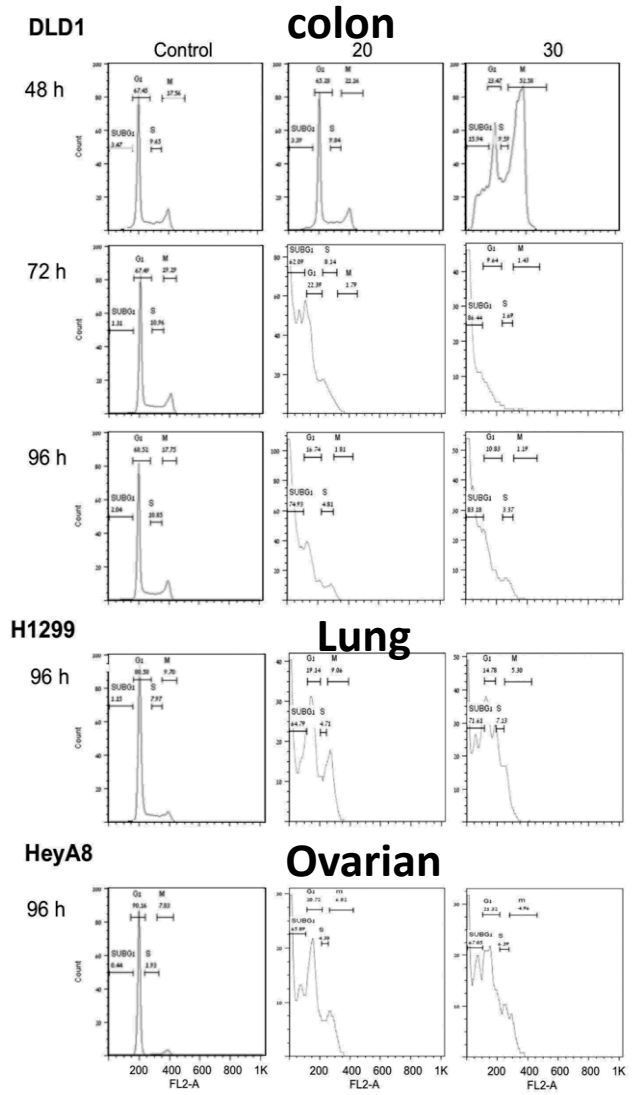
Castiel et al., BMC Cancer, 2011

Inbar-Rozensal et al, Breast. Canc. Res., 2009

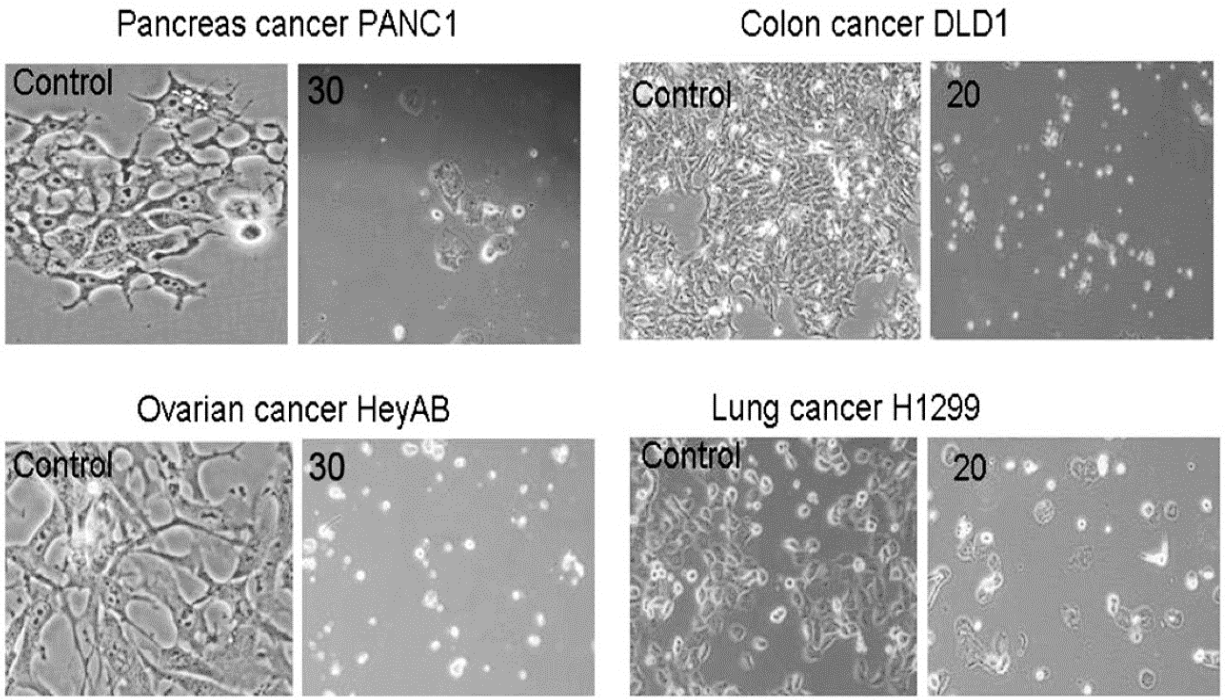
PJ34 causes Mitosis Arrest and Cell Death in human cancer cells measured by flow-cytometry

Mitosis Arrest and cell death in human cancer cells treated with the indicated [PJ34] (μM) for 96 hours in the indicated cells:

Pancreas- PDAC, PANC1, Colon -DLD1, Lung H1299, Ovary HeyA8

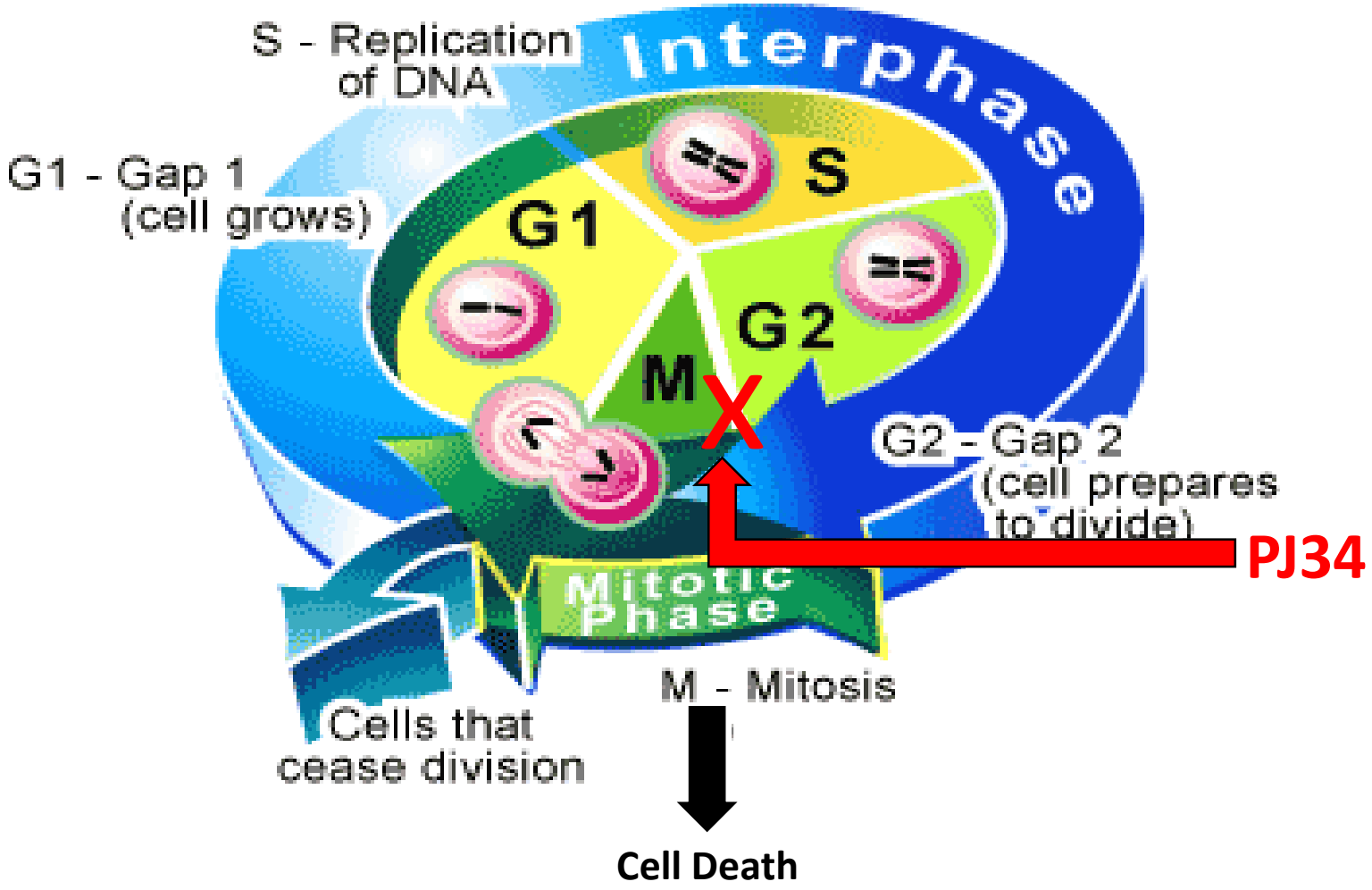


96 hours incubation with PJ34

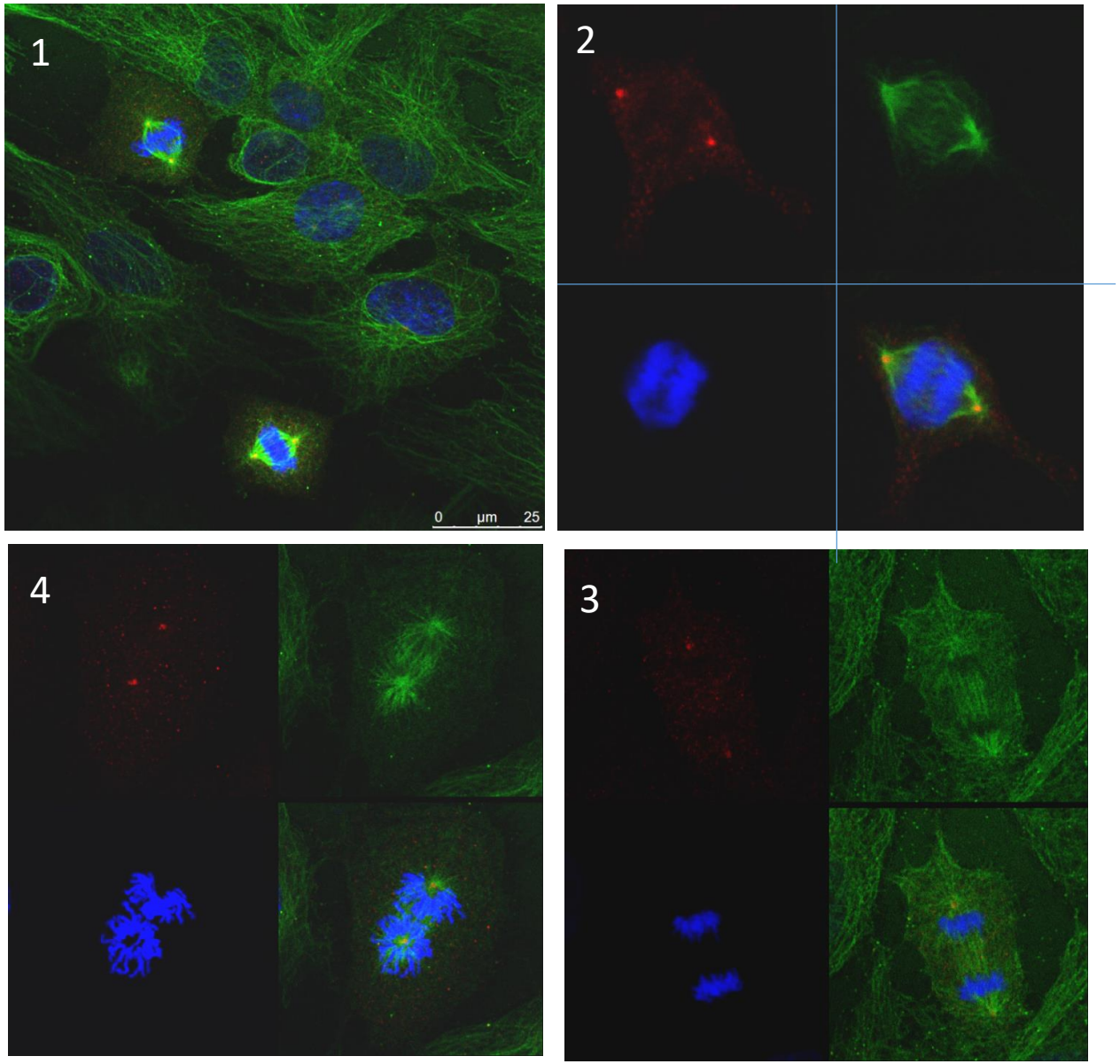


Castiel et al., BMC Cancer, 2011

PJ34 Arrests Mitosis and Induces Cell Death in Human Cancer Cells



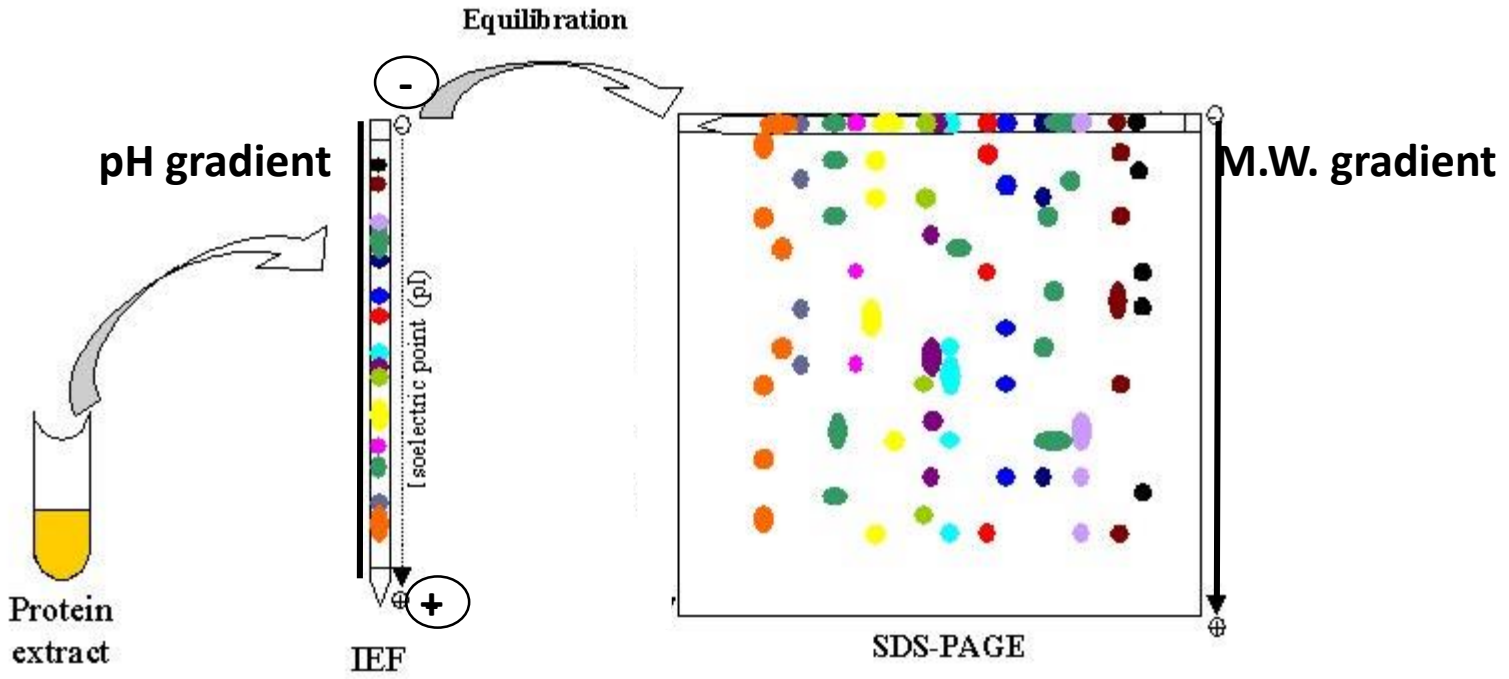
Mitosis in Non-malignant epithelial cells treated with PJ-34 20 µM



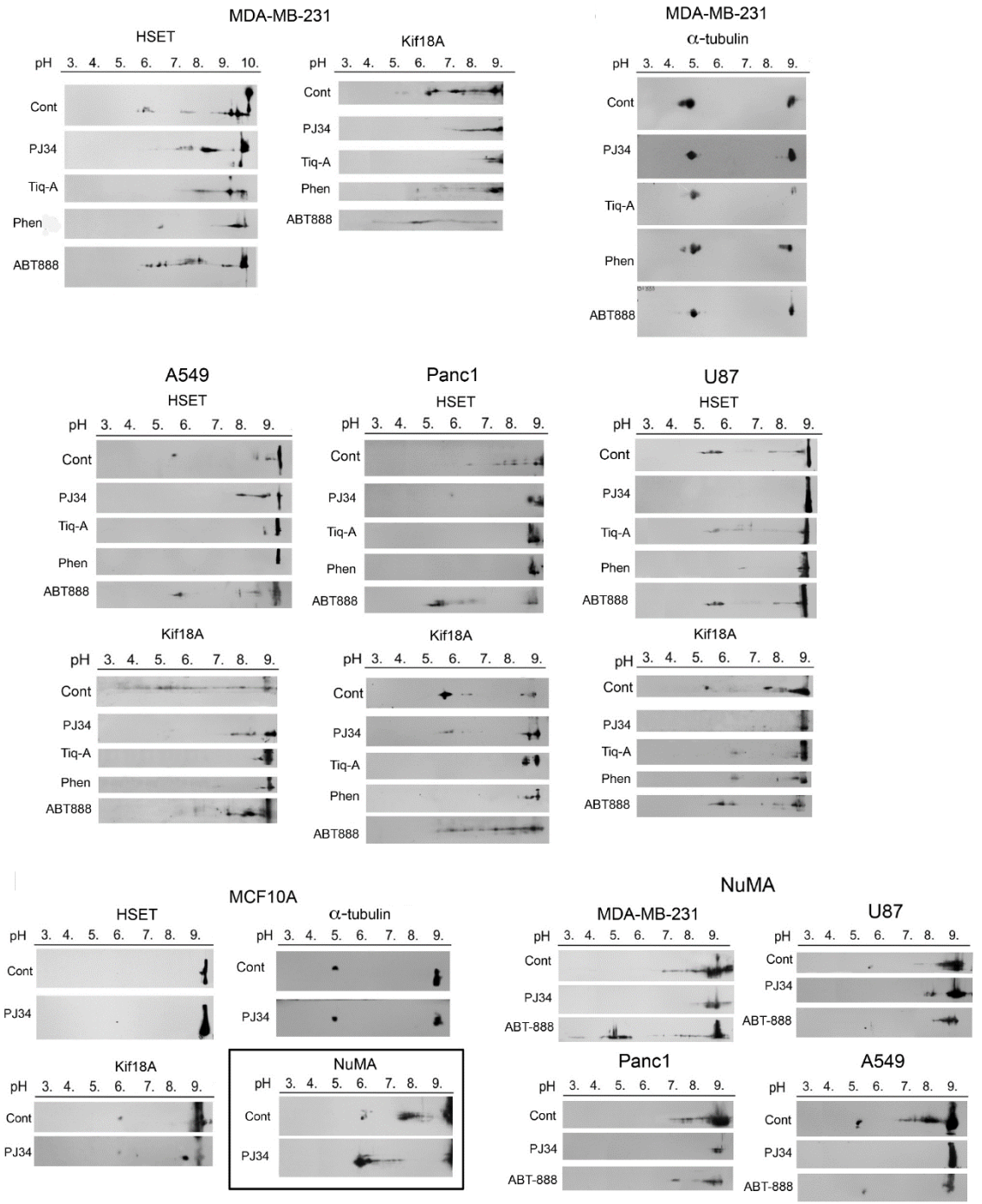
microtubles, centrosomes, chromosomes

Castiel et al., BMC Cancer 11, 412, 2011

Measuring changes in the post-translational modifications of proteins by the shift in their isoelectric point on pH gradient (2-D gels)



The Phenanthridine PJ34 interfered with the PTM of NuMA and kinesins kifC1/HSET and kif18A only in cancer epithelial cells: Pancreas PANC1, breast TN MDA-MB-231, lung A549, and glioblastoma U87.
Other modified phenanthridines, Tiq-A and Phen had a similar but milder effect. A non-phenenthridine yet a PARP inhibitor, ABT-888, lacks this effect.

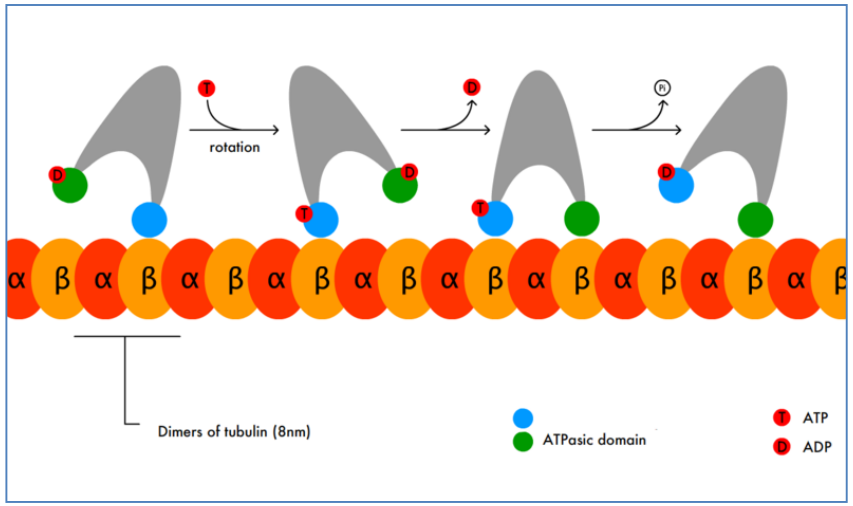


Cohen-Armon, Drug Dis. today, 2022
Cohen-Armon, cancers, 2020
Visochek et al., Oncotarget 2017

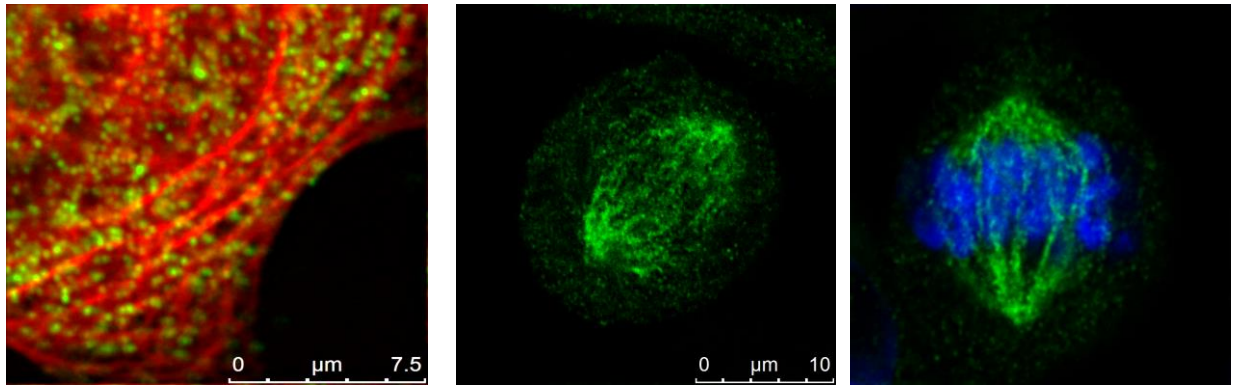
Kinesin HSET/kifC1 is implicated in the construction of microtubules in the mitotic spindle

Kinesin Kif18A is implicated in the attachment of chromosomes to microtubules in the spindle mid-zone

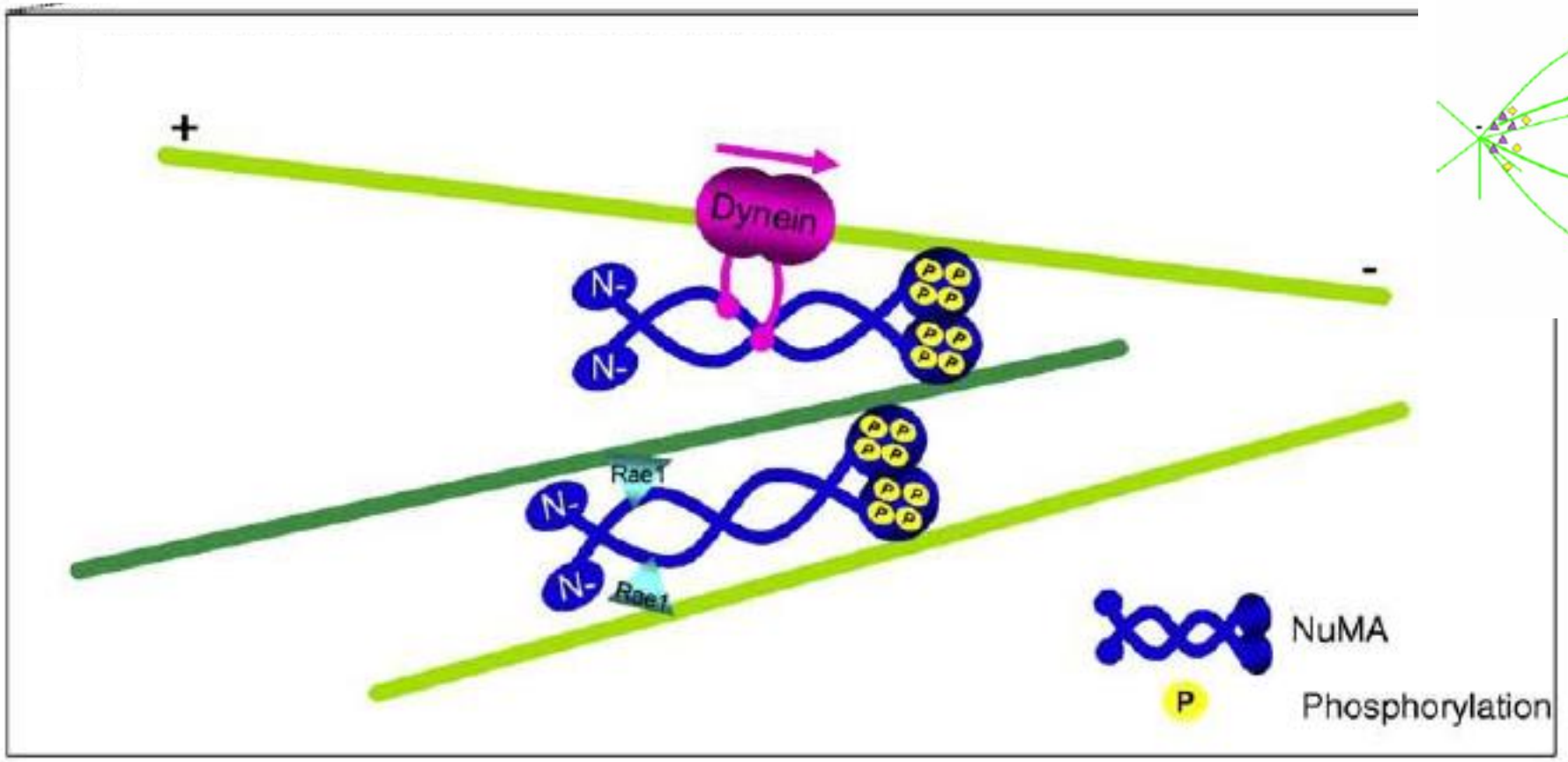
Kinesin sliding on microtubules

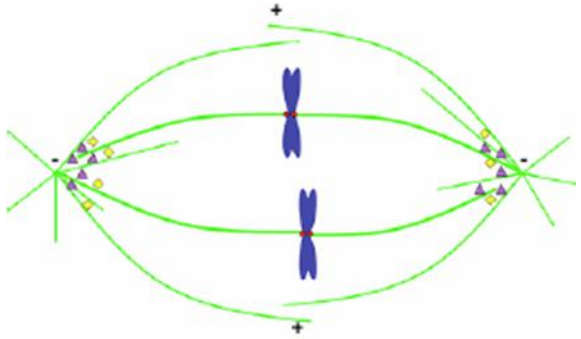


α-tubulin in the microtubules (red);
HSET/kinesinKifC1/Kif14 (green)
chromosomes (blue)



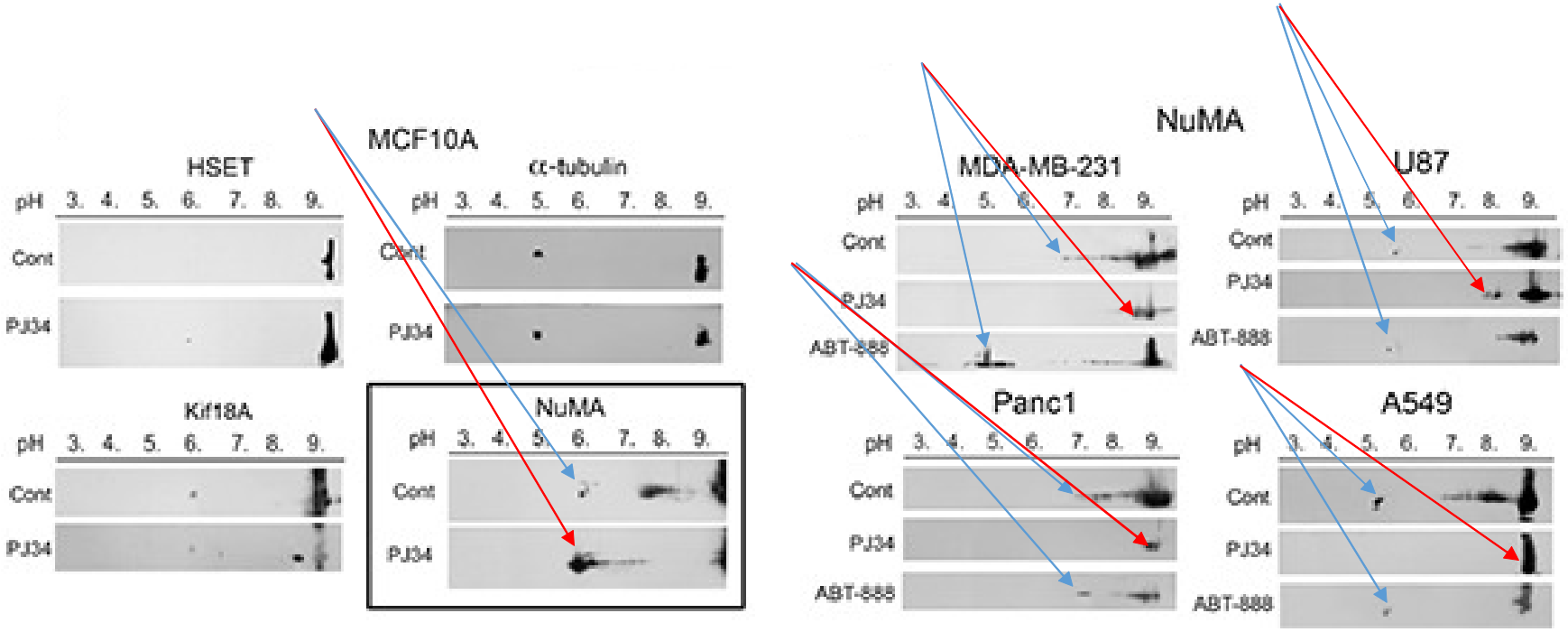
NuMA binding to proteins is crucial for its indispensable function in the mitotic spindle



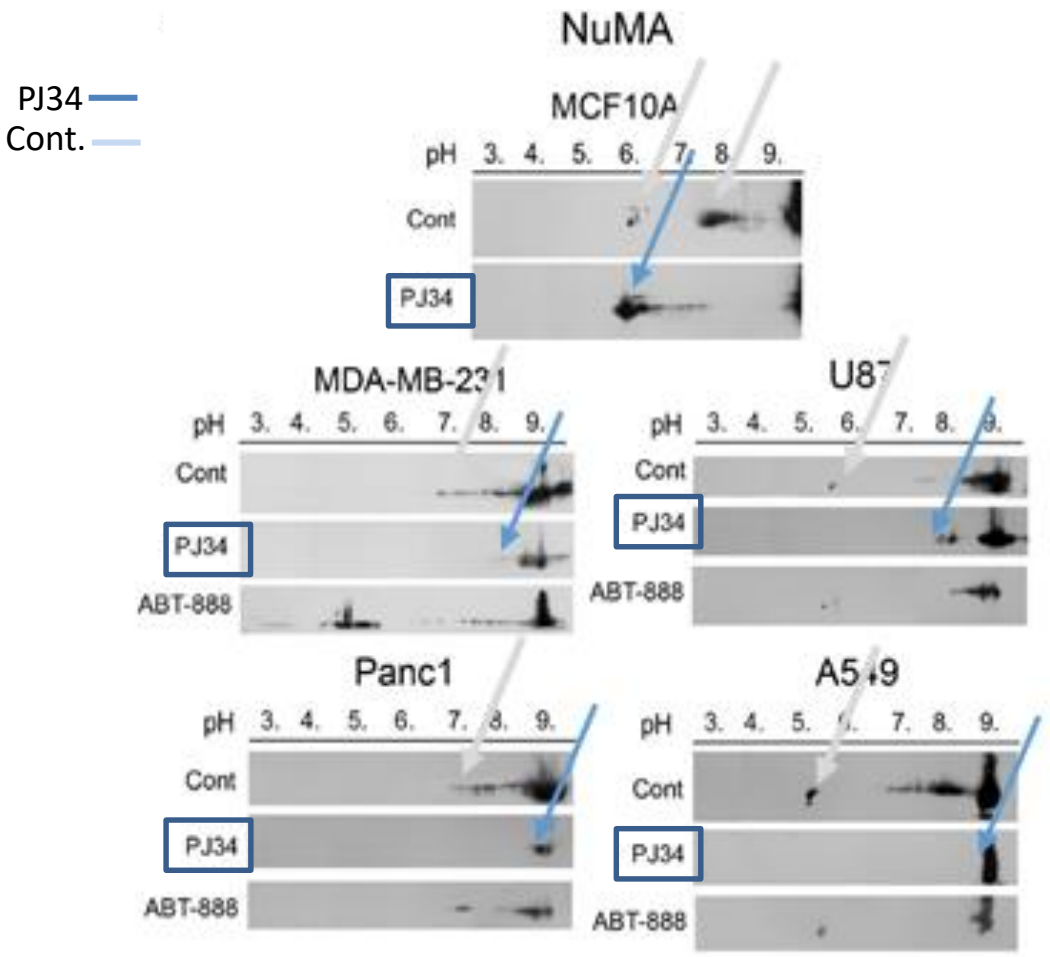


From: Radulescu and Cleveland Trends Cell Biol., 2010, 20: 214-222

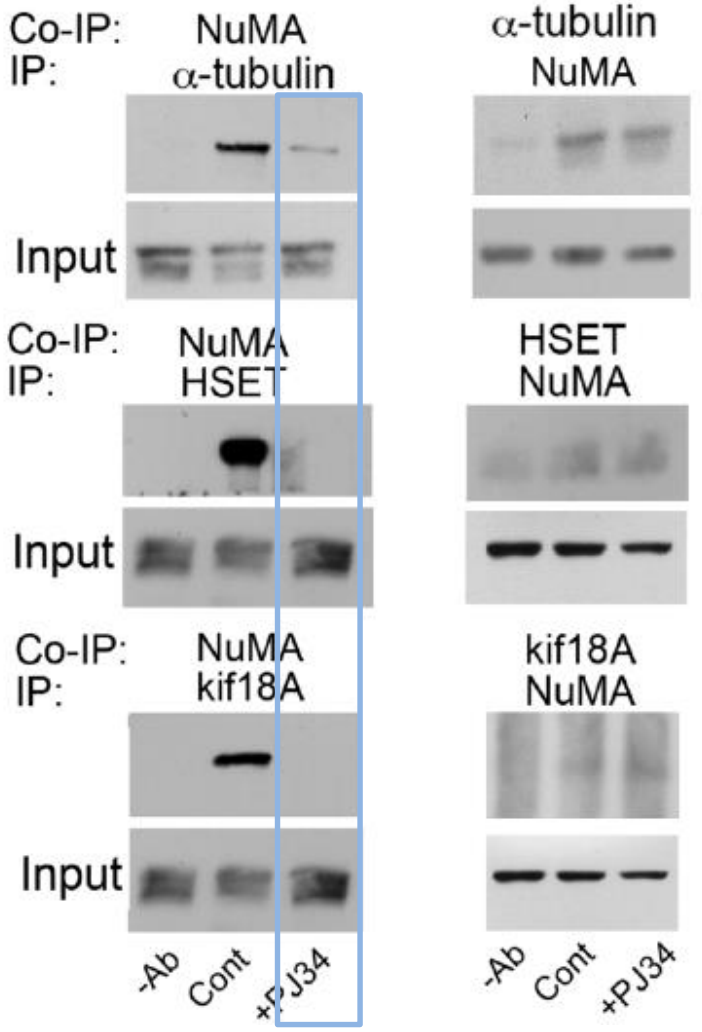
The post translational modification of NuMA is inhibited in epithelial cancer cells



PJ34 exclusively prevents the PTM of NuMA in human cancer cells: PDAC PANC1, breast TN MDA-MB-231, lung A549, and glioblastoma U87

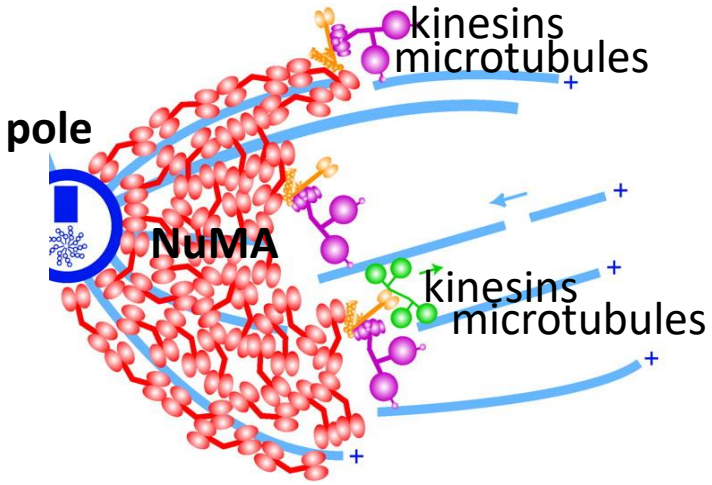


Treatment with PJ34 exclusively prevents the co-immuno-precipitation of NuMA with proteins in cancer cells



Cohen-Armon, Drug Dis. today, 2022
Cohen-Armon, cancers, 2020
Visochek et al., Oncotarget, 2017

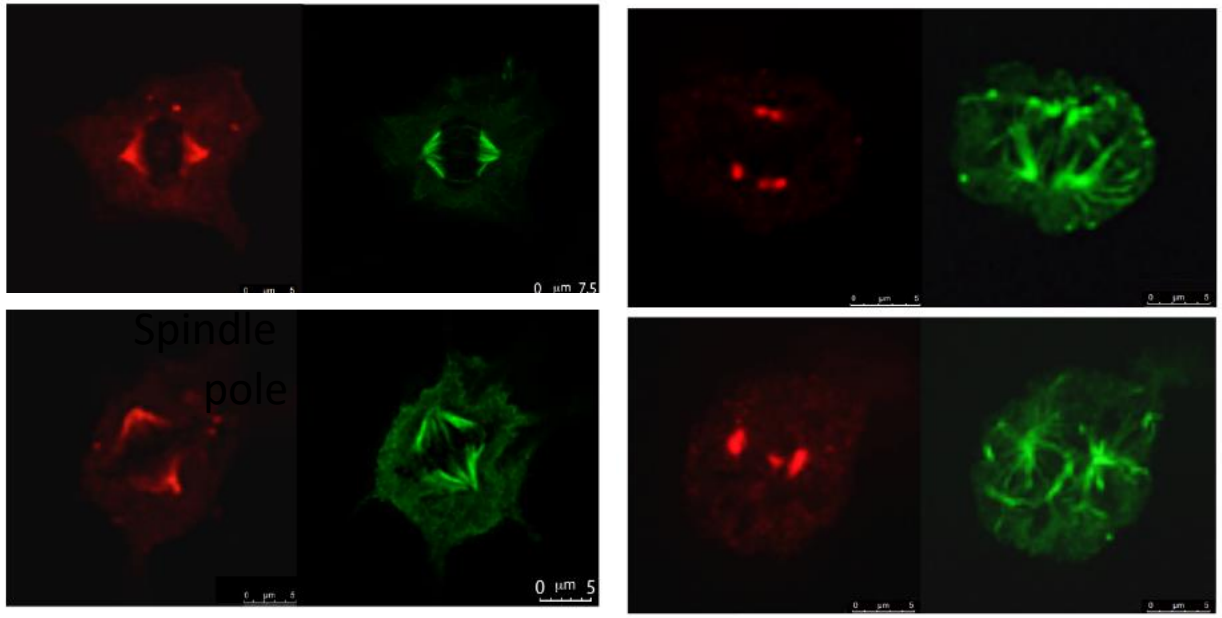
Aberrant spindles with un-clustered NuMA in the spindle poles in human cancer cells treated with PJ34



Treatment with PJ34

Healthy breast epithelial cells — MCF10A

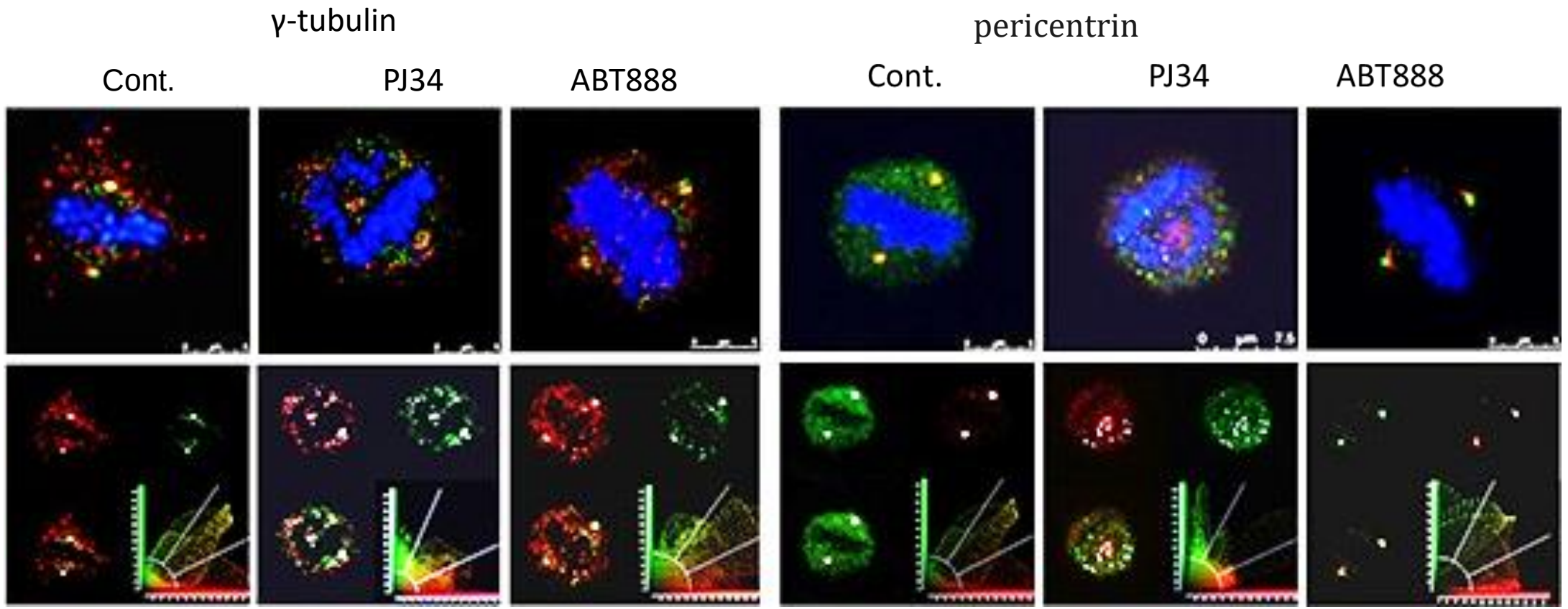
Breast malignant epithelial cell — MDA-MB-231



α-tubulin (microtubules)
NuMA

Visochek et al., Oncotarget, 2017

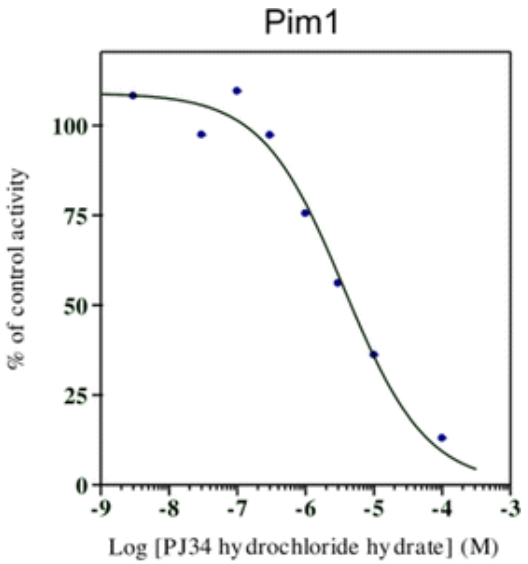
Disrupted spindle poles with dispersed chromosomes and dispersion of tankyrase1 with γ-tubulin and pericentrin in spindles of multi-centrosomal MDA-MB-231 triple-negative breast cancer cells treated with PJ34

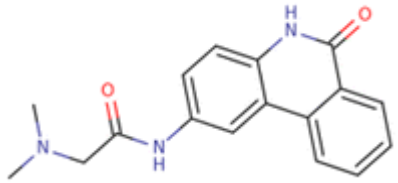


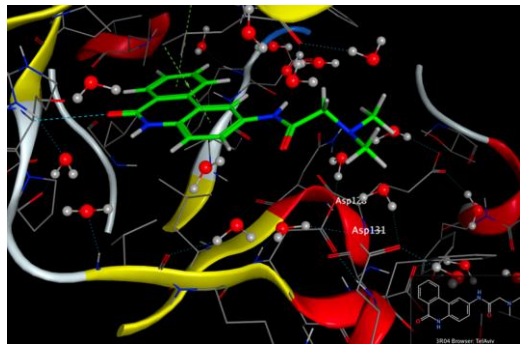
tankyrase1
γ-tubulin
Pericentrin
Chromosomes

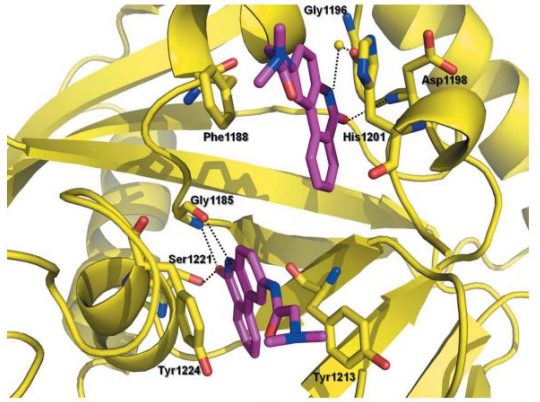
Visochek et al Oncotarget, 2017

PJ34 inhibits the activity of the kinase pim1 and of tankyrase1, both modify NuMA in human cancer cells and promote its protein-binding capacity









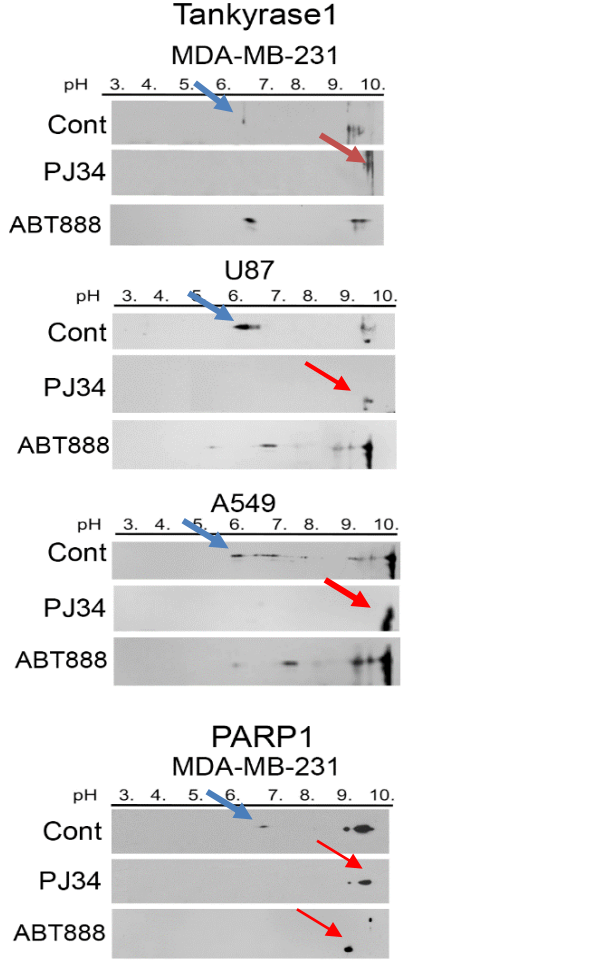
IC_{50}=3.7 μM

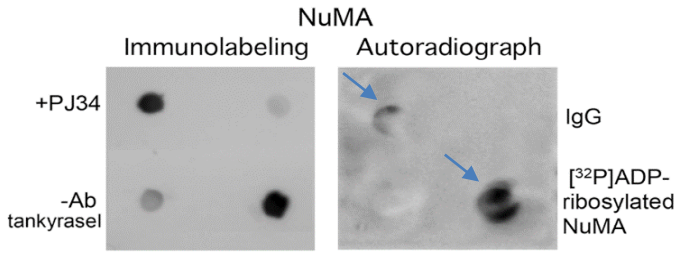
IC_{50}=1. μM

Antolin AA., et al., ACS Chem Biol., 2012

Kirby CA., et al. Acta Cryst, 2012

Tankyrase1 and NuMA polyADP-ribosylation



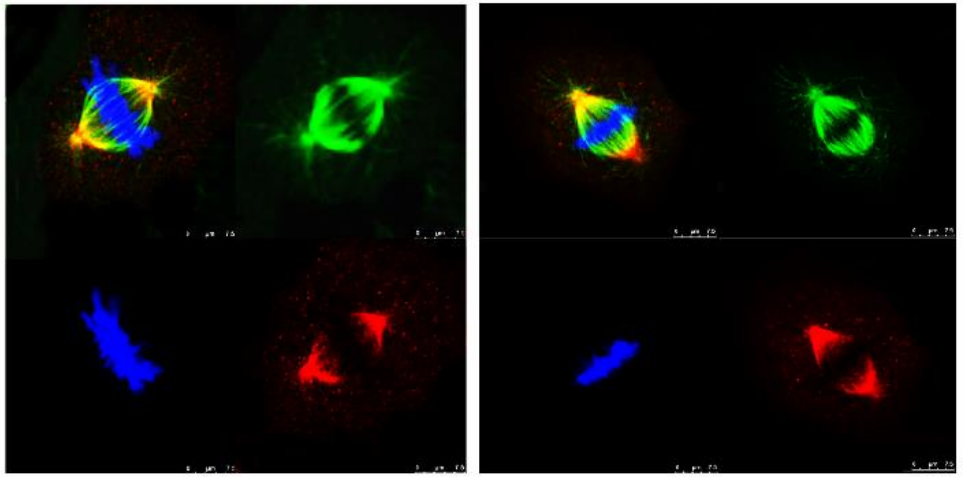


Visochek et al., Oncotarget, 2017

Aberrant spindle poles and dispersed NuMA, centrosomes and chromosomes in multi-centrosomal TN breast cancer cells MDA-MB-231 treated with PJ34

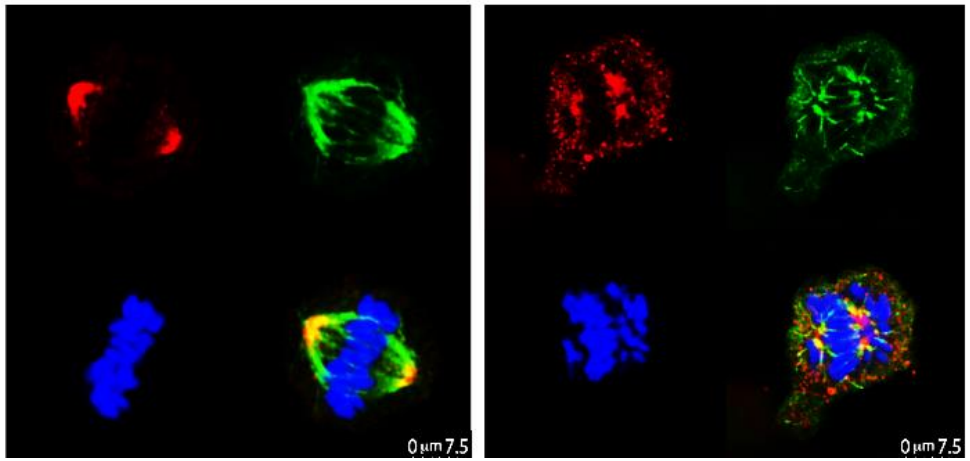
MCF10A

Control PJ34



MDA-MB-231

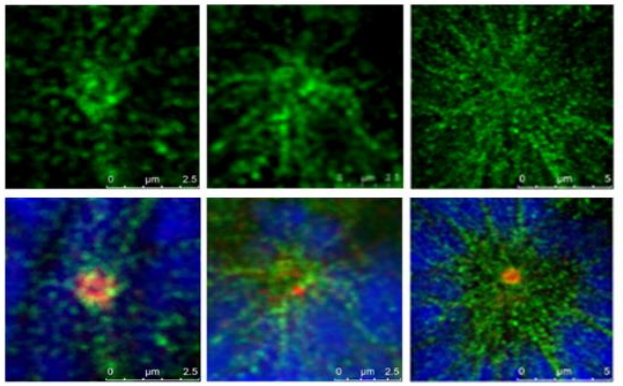
Control PJ34



α-tubulin (microtubules)
Chromosomes
NuMA

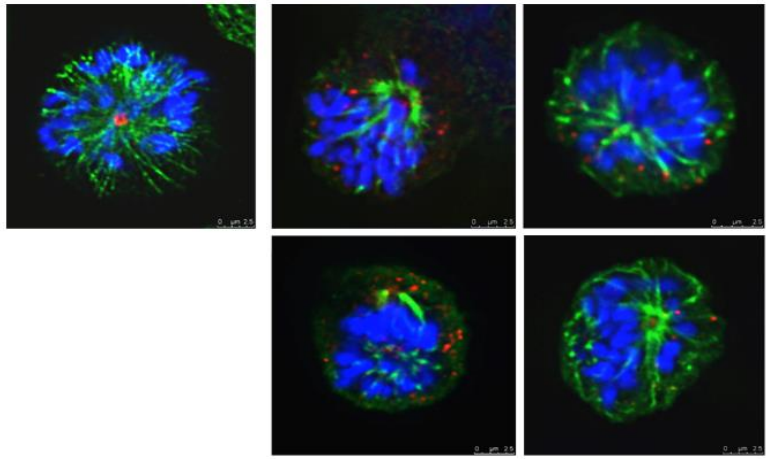
MCF10A

Control PJ34



MDA-MB-231

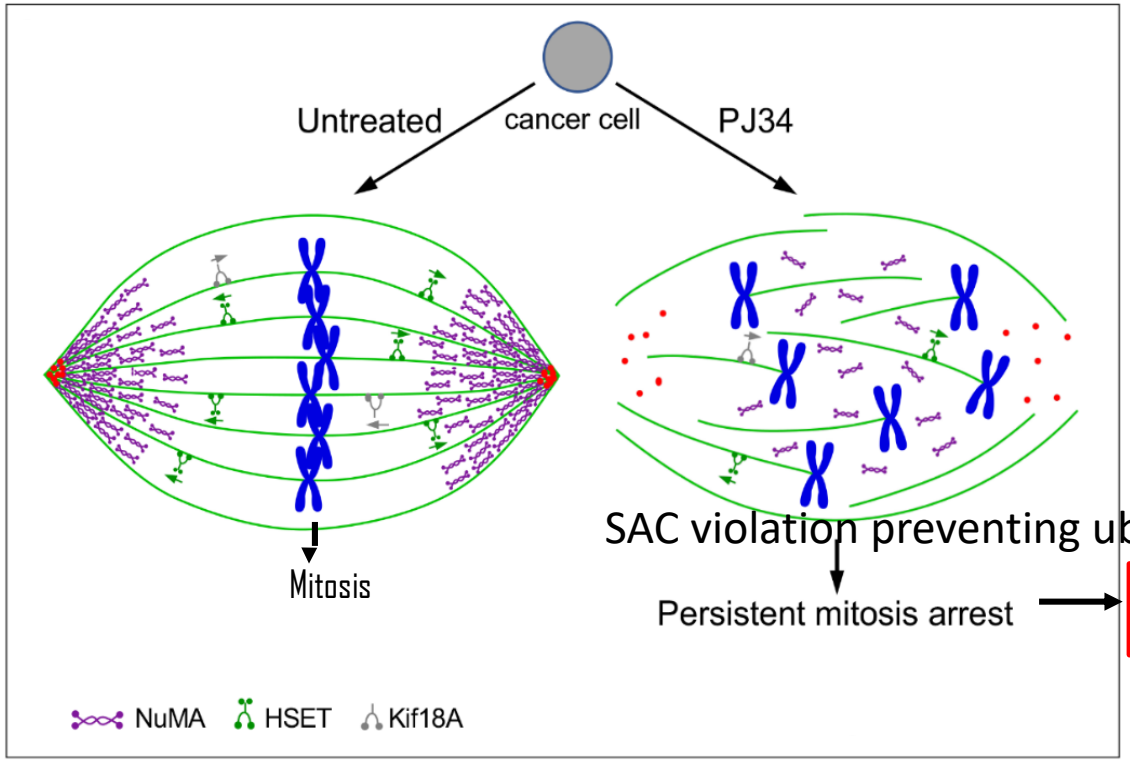
Control PJ34

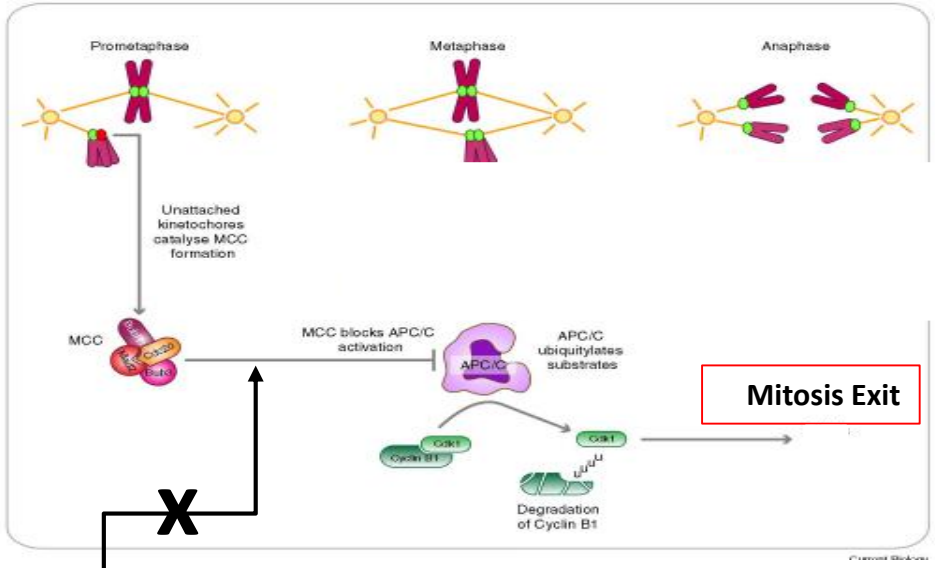


kinesinC1/HSET (microtubules)
Chromosomes
γ-tubulin (Centrosomes)

Visochek et al., 2017

NuMA un-clustering in the spindle poles causing aberrant spindles with dispersed centrosomes and chromosomes lead to mitotic arrest in cancer cells treated with PJ34





Lara-Gonzales et al., 2012 Cur. Biol.

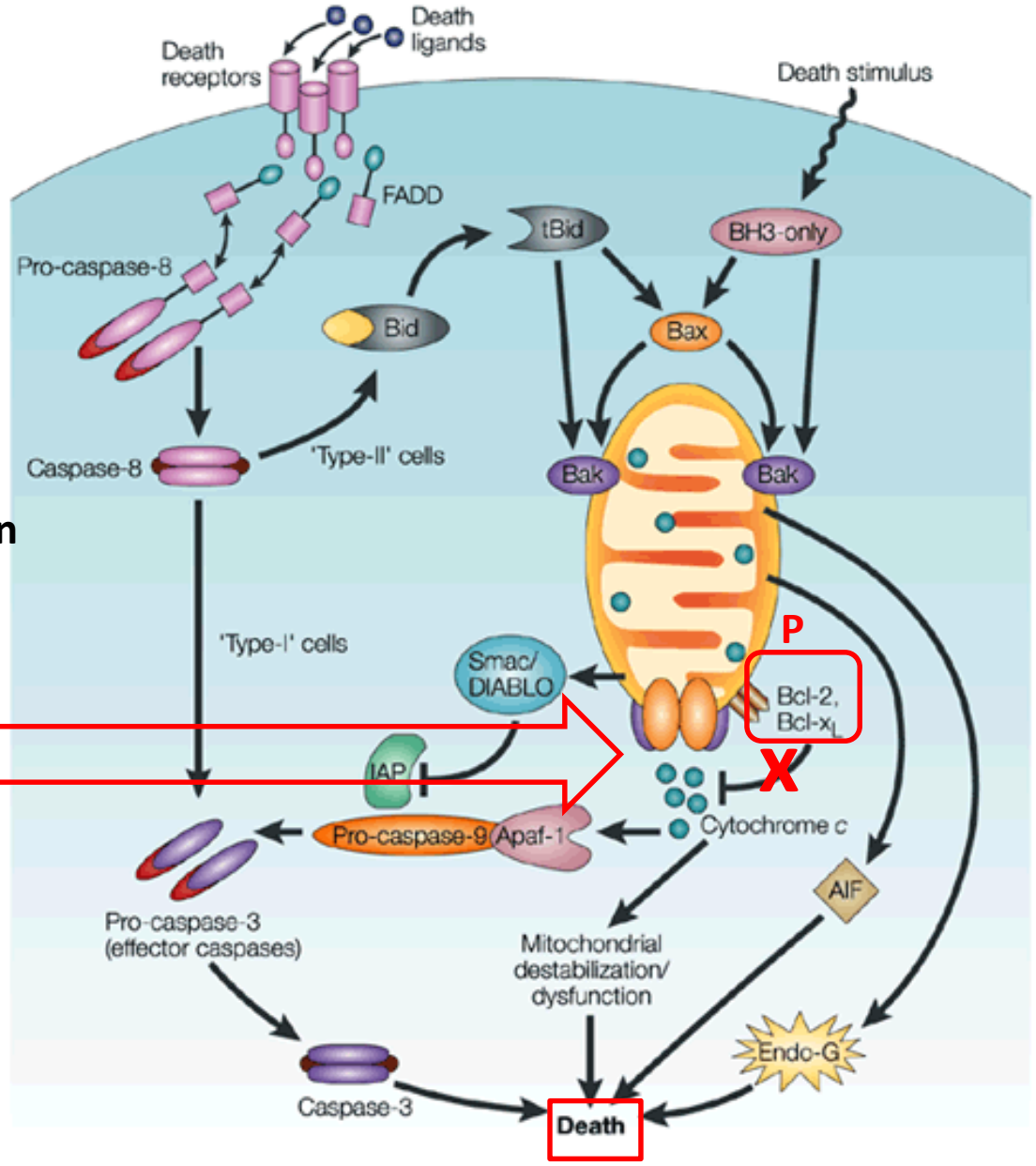
SAC violation preventing ubiquitination of cyclinB1

Persistent CyclinB1 activation of CDK

Cohen-Armon, Drug Dis. today, 2022
Cohen-Armon, cancers, 2020

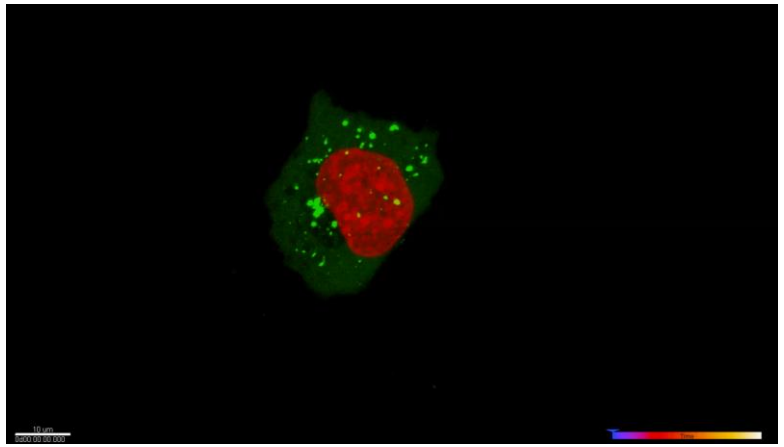
Mitosis arrest leads to
Mitotic Catastrophe cell death

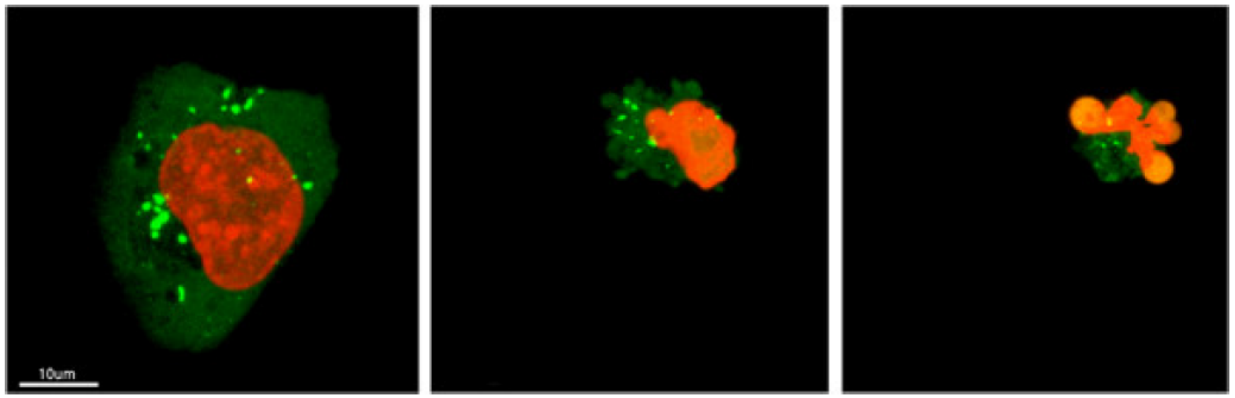
Bcl-2 and Bcl_{xL} phosphorylation by CyclinB1-activated CDK causes Cytochrome-C leakage and Cell Death



Nature Reviews | Molecular Cell Biology

Cell-death during mitosis in PJ34-treated extra-centrosomal breast malignant cells TN MDA-MB-231, documented at real time by Confocal imaging. Un-clustered centrosomes and dispersed chromosomes in the cells transfected with GFP-γ-tubulin (green) and with H2B-red.





Castiel et al, JOVE, 2013

Summary

- First evidence for an exclusive modification of NuMA in cancer cells causing cell death

- A treatment causing self-eradication of malignant cells during mitosis, while healthy proliferating cells and quiescent cells are spared.
- No adverse effects were observed in nude mice, as well.

- In this mechanism, cancer cells are eradicated regardless of their genetic mutations. The more rapidly they proliferate, the more rapidly they are eradicated.

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NOVARTIS

Israel Cancer Research Fund

TAU Am. Friends

TEL AVIV UNIVERSITY
Pursuing the Unknown

TAU-Sheba Medical center collaboration Fund

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Thank you