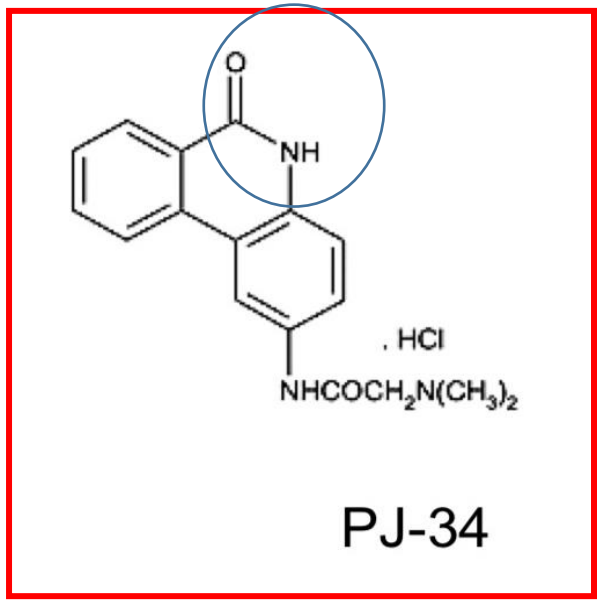
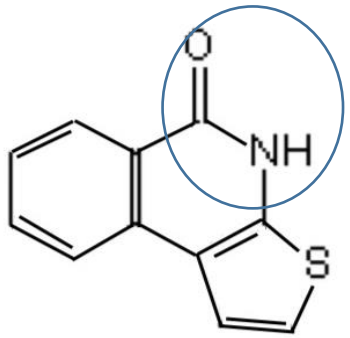
The Modified Phenanthridine PJ34 Unveils a Cell-Death Mechanism Exclusive to Human Cancer Cells

Prof. M. Cohen-Armon
Sackler School of Medicine and Sagol School of Neuroscience
Tel-Aviv University

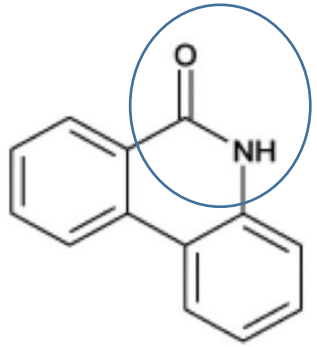
Phenanthrene derivatives



PJ-34

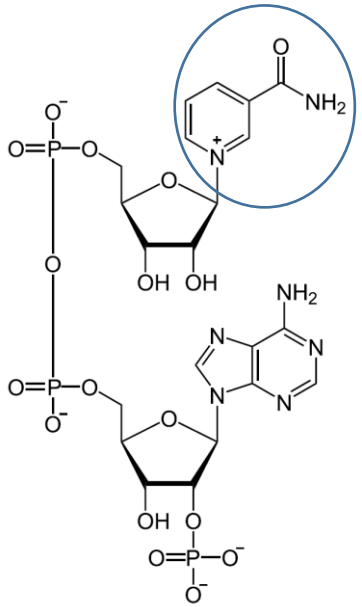


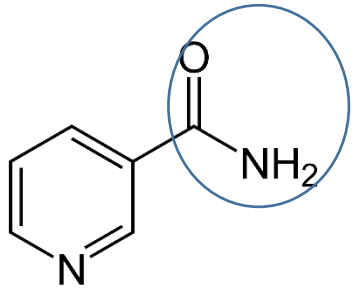
Tiq-A



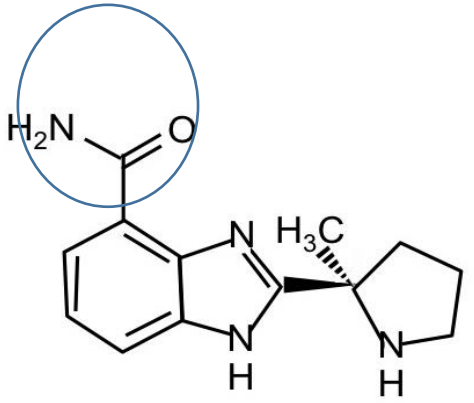
Phen

NAD





Nicotinamide



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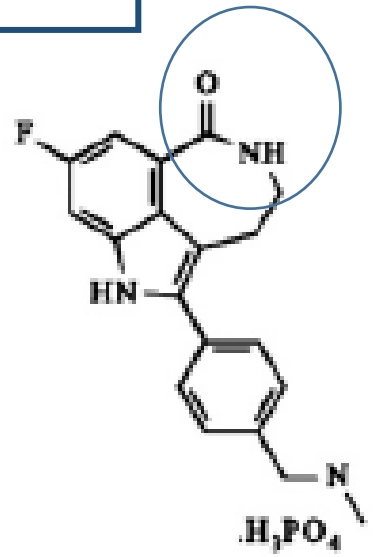


AG014699

Tricyclic molecules eradicating human cancer cells

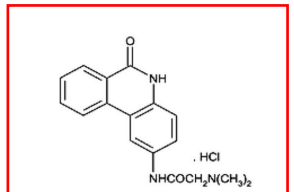
Castiel et al. BMC Cancer 2011, 11:412
http://www.biomedcentral.com/1471-2407/11/412

BMC Cancer

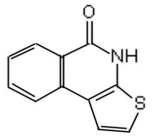
RESEARCH ARTICLE Open Access

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

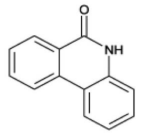
Asher Castiel[4†], Leonid Visochek[1†], Leonid Mittelman[3], Françoise Dantzer[5], Shai Izraeli[2,4] and Malka Cohen-Armon[1*]



PJ-34



Tiq-A

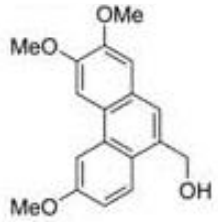


Phen

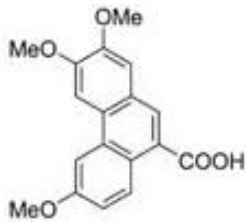
Cancer Science
The official journal of the Japanese Cancer Association
JCA

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

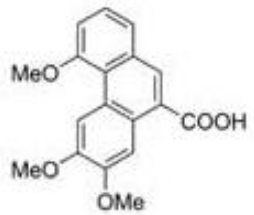
Ying-Ying Wang,[1] Tsuyoshi Taniguchi,[2] Tomohisa Baba,[1] Ying-Yi Li,[1,3,4] Hiroyuki Ishibashi[2] and Naofumi Mukaida[1,5]



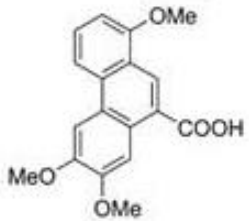
No.5



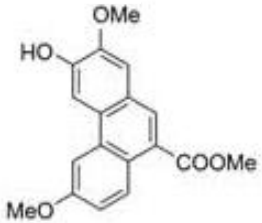
T19



T19-1



T19-2

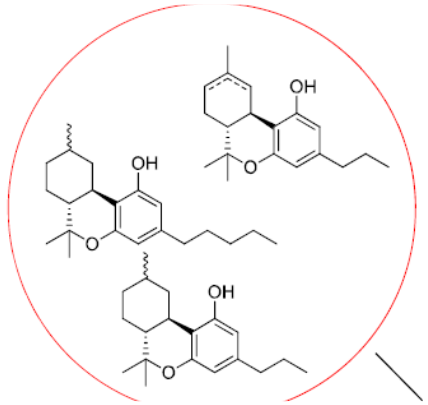


T26

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*

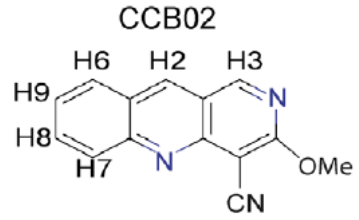


TRANSPARENT PROCESS
OPEN ACCESS
THE EMBO JOURNAL

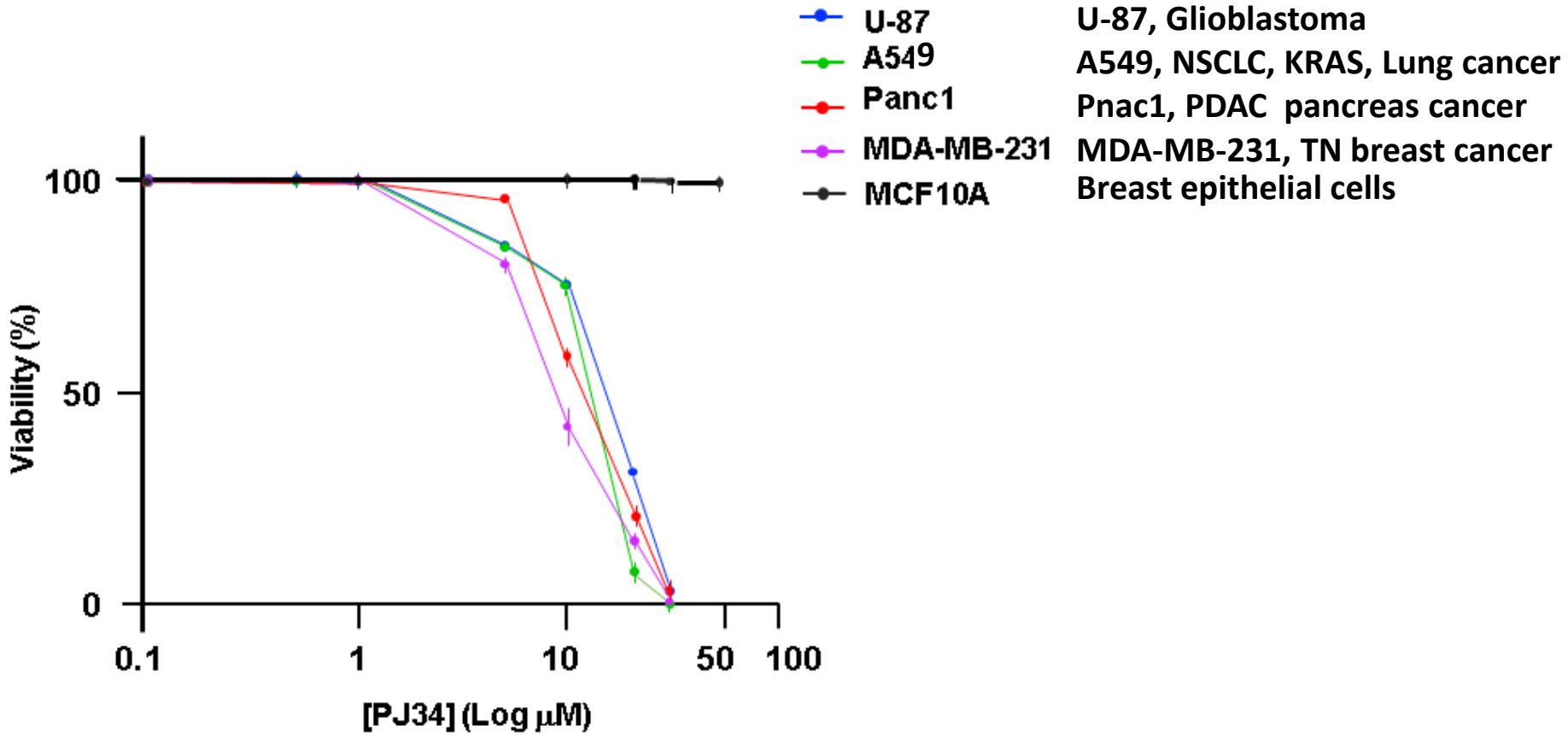
Article

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,*]



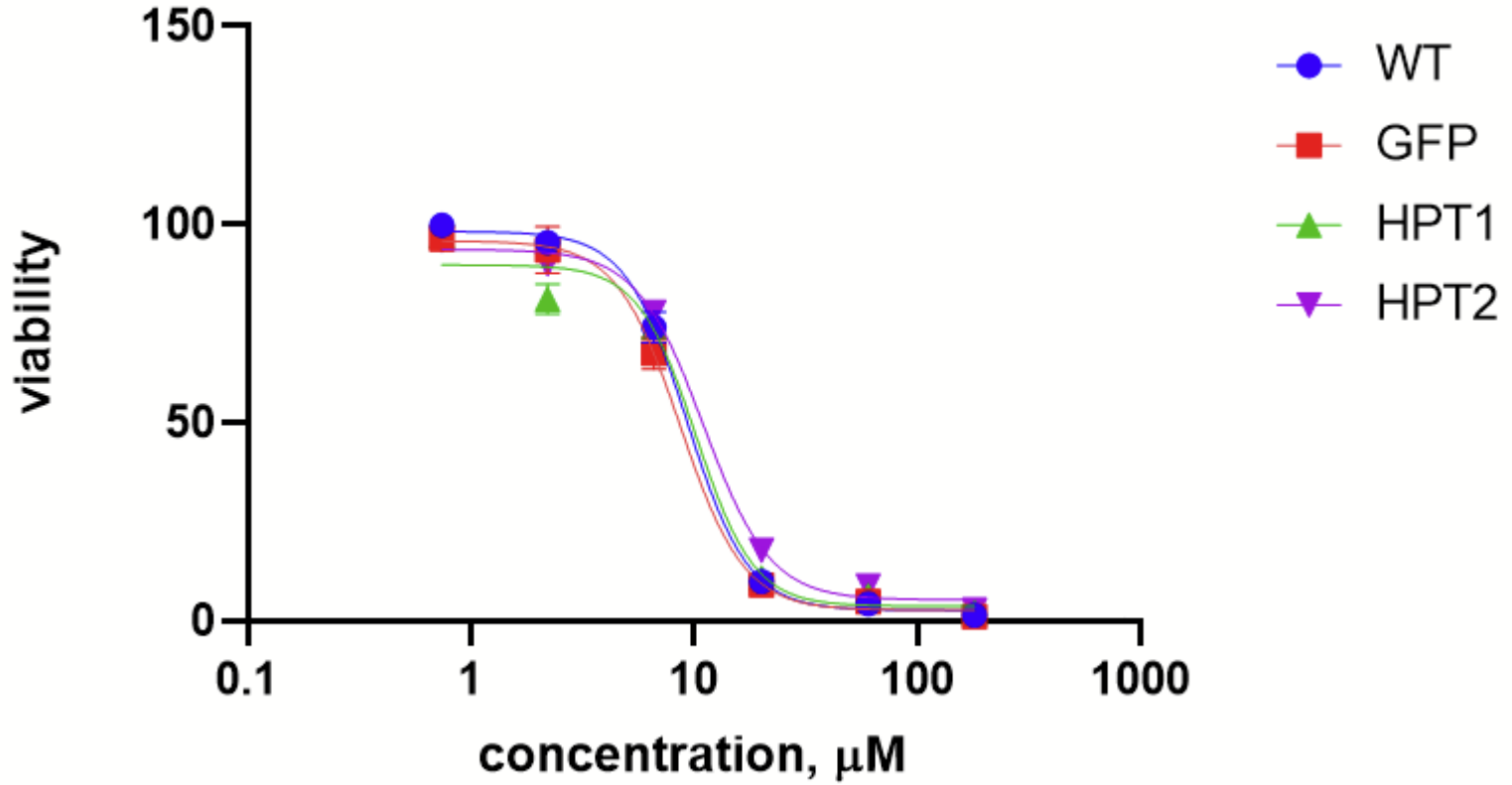
Exclusive eradication of the indicated human malignant epithelial cells treated with PJ34 (96 hours) at the indicated concentrations. Benign human breast epithelial cells are not impaired.



Cohen-Armon, Drug dus. Today, 2022

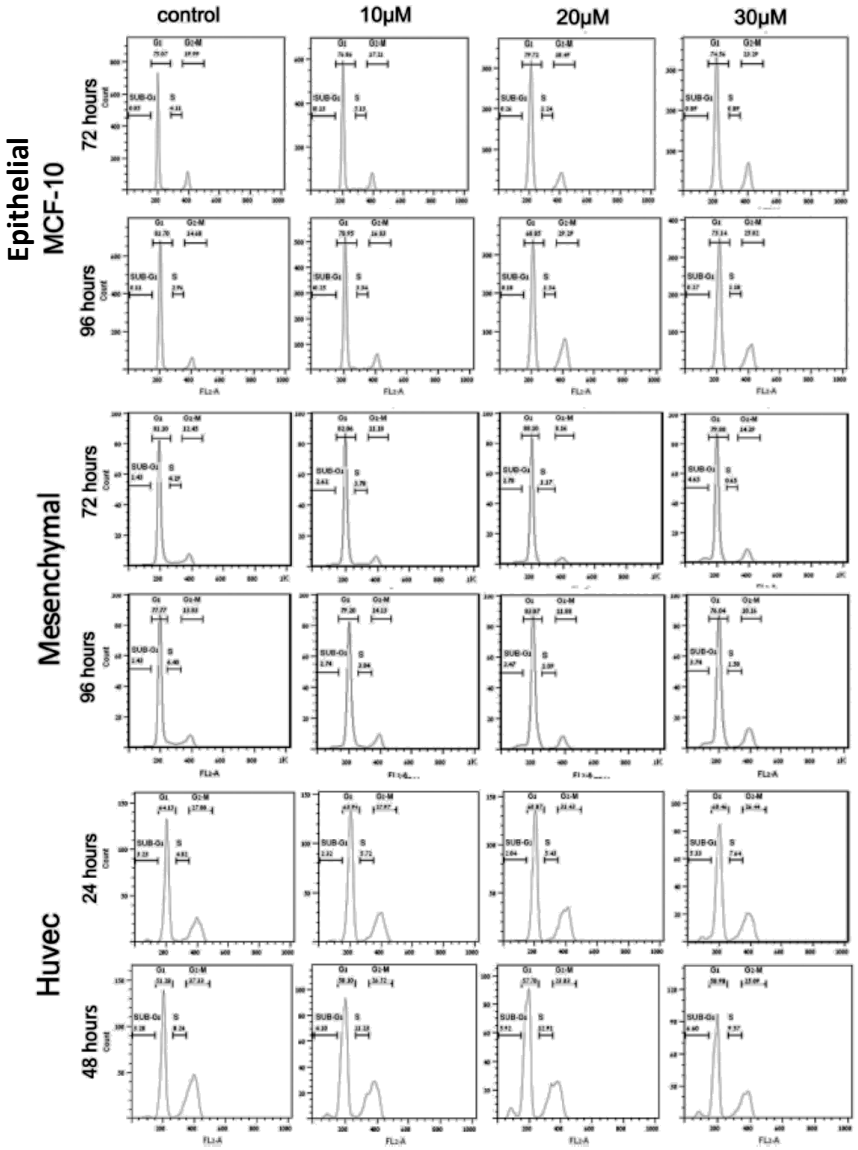
PJ34 dose-dependently eradicates human colorectal carcinoma cell line HCT116

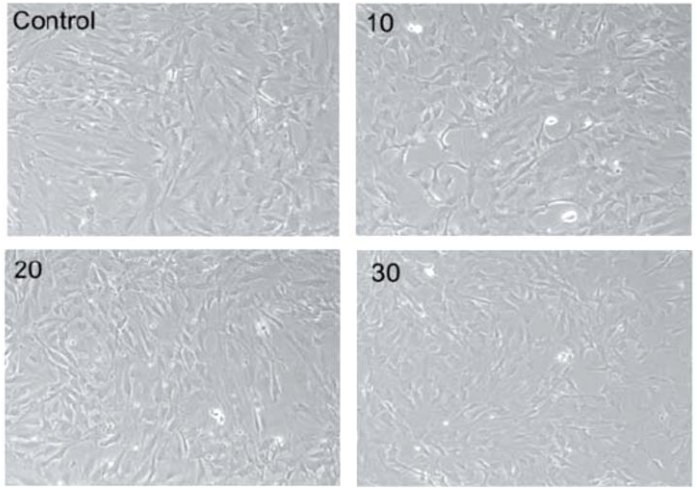
Both Near-diploid and highly- aneuploid cancer cell lines



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

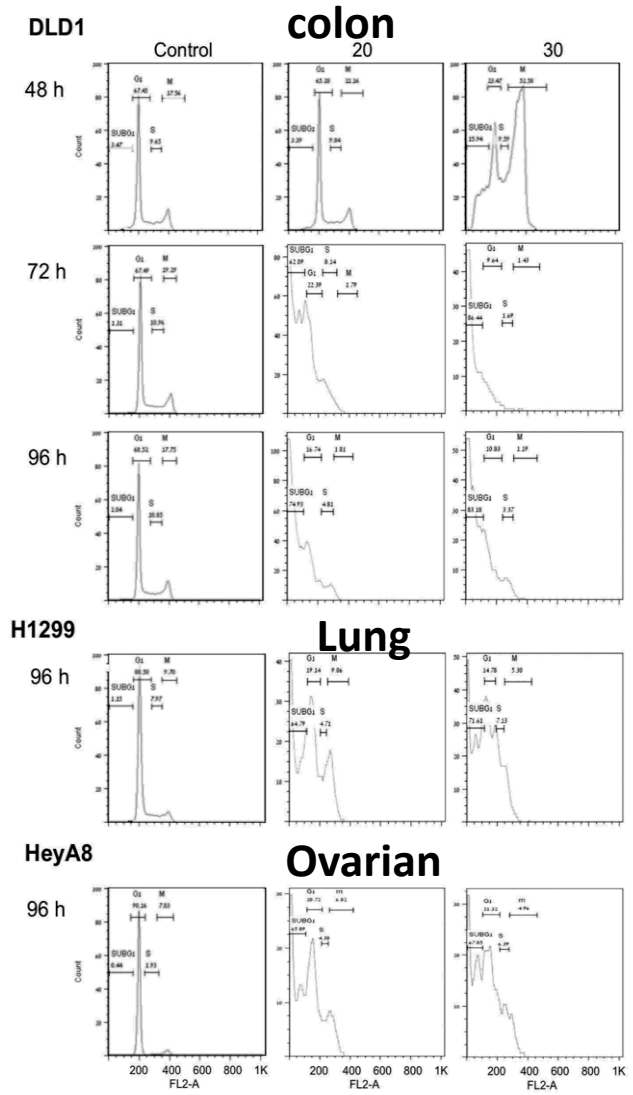
Castiel et al., BMC Cancer, 2011

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

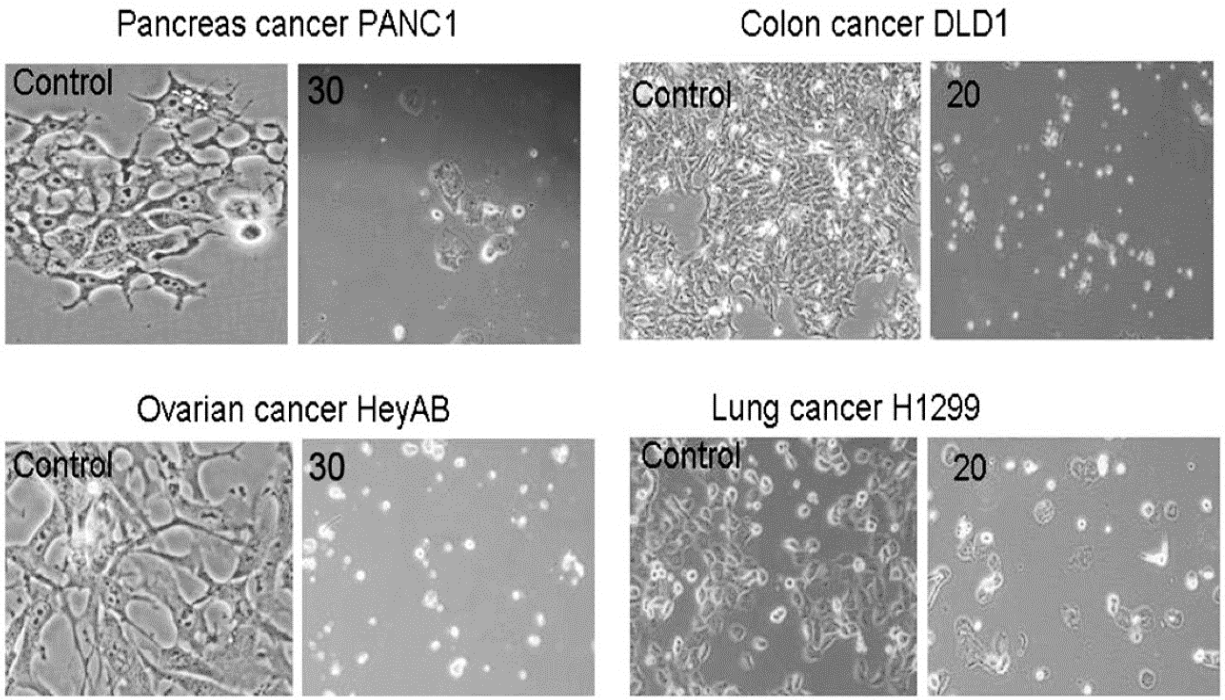
PJ34 causes Mitosis Arrest and Cell Death in human cancer cells measured by flow-cytometry

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

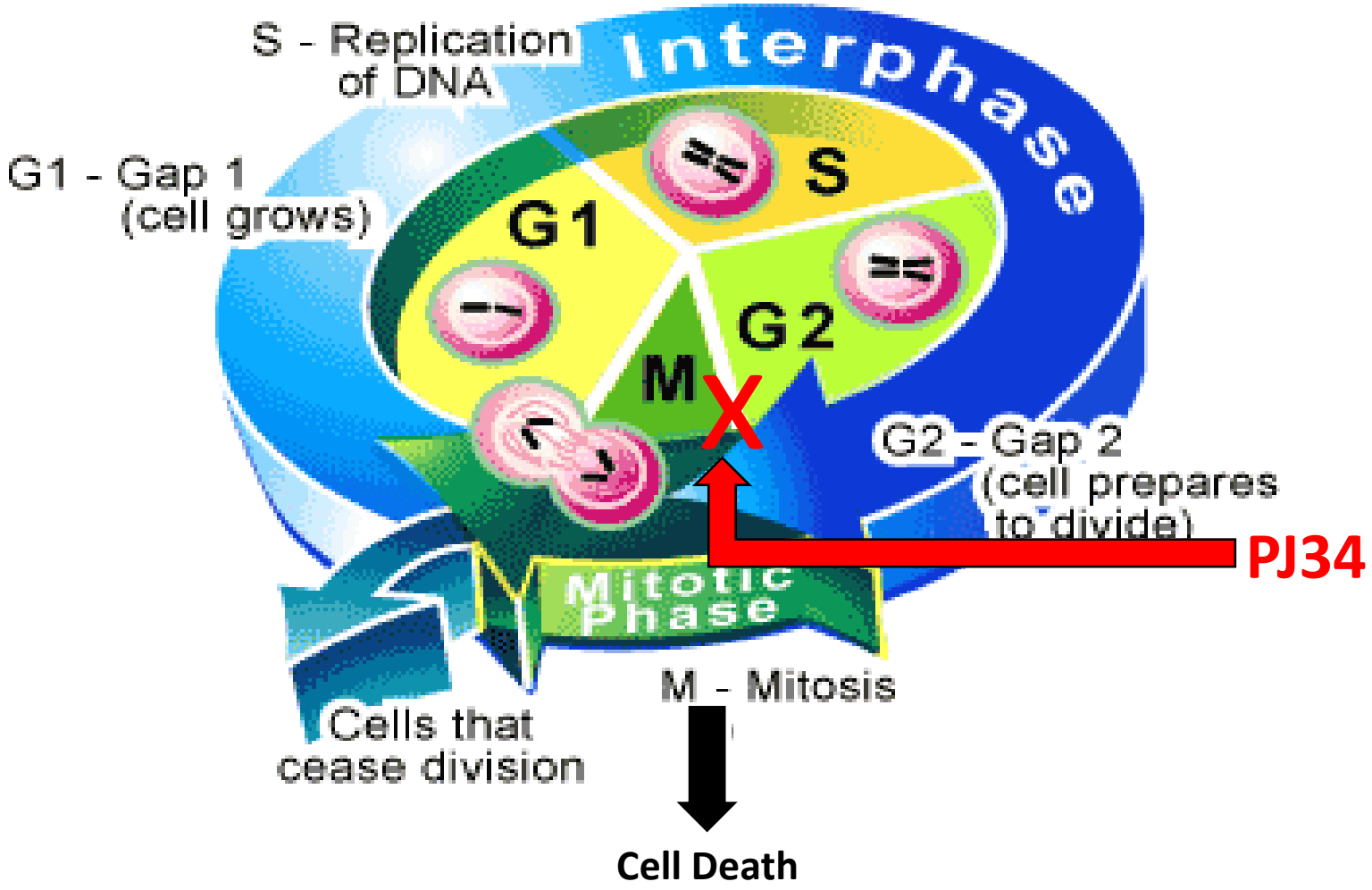


96 hours incubation with PJ34

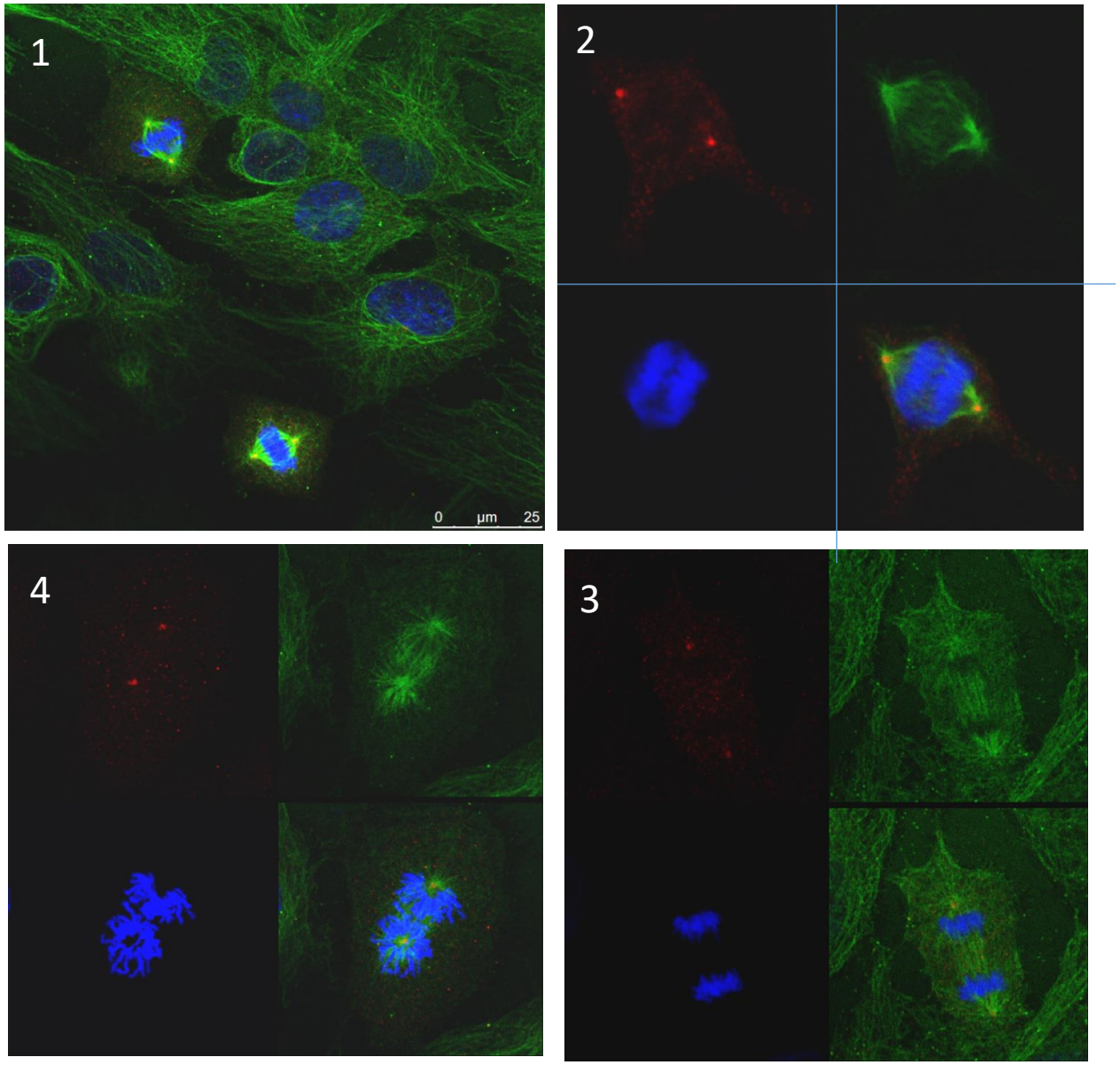


Castiel et al., BMC Cancer, 2011

PJ34 Arrests Mitosis and Induces Cell Death in Human Cancer Cells



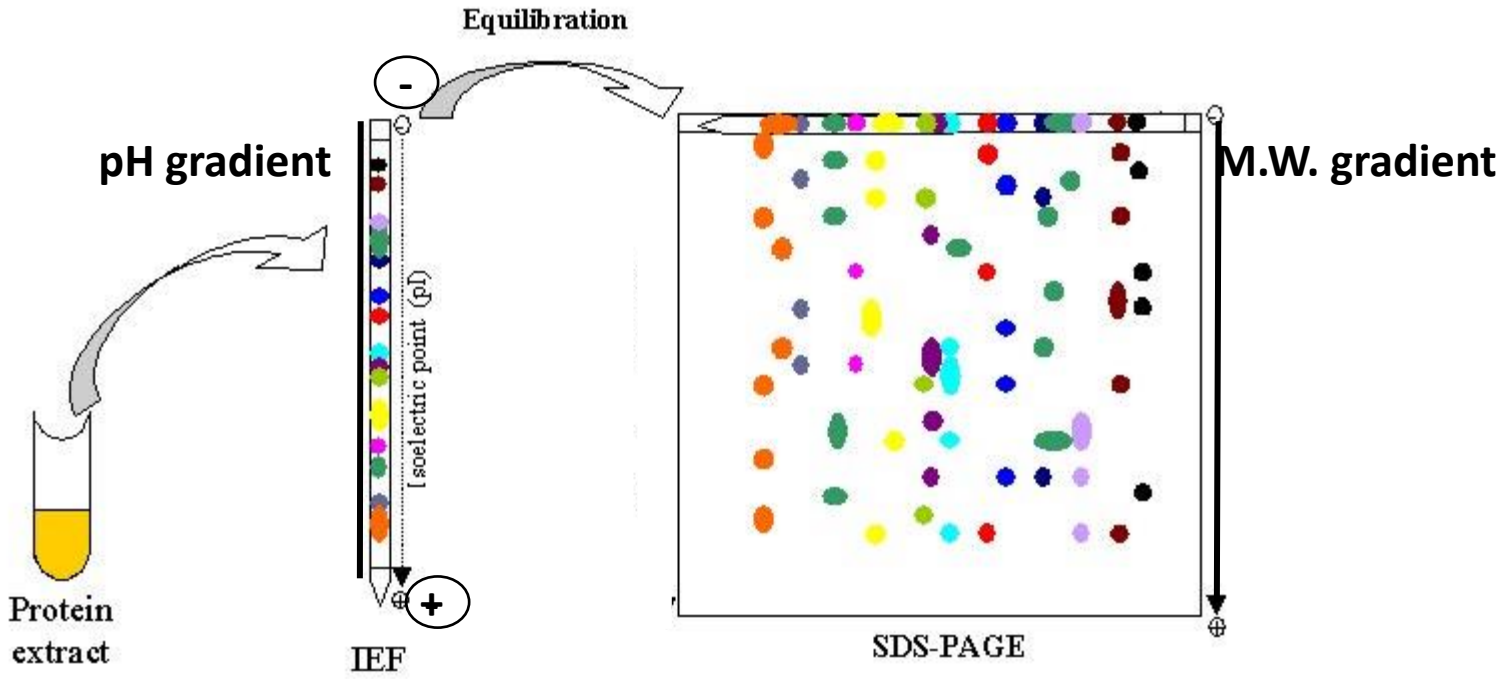
Mitosis in Non-malignant epithelial cells treated with PJ-34 20 µM



microtubles, centrosomes, chromosomes

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

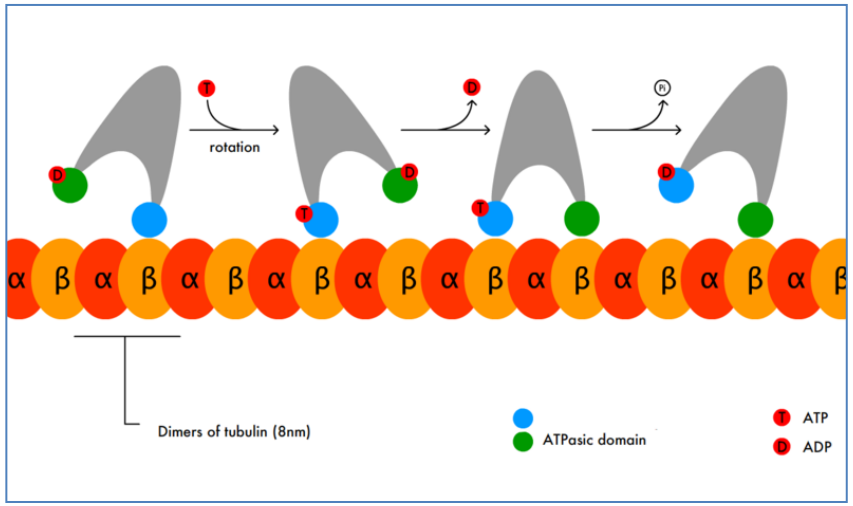
Measuring changes in the post-translational modifications of proteins by the shift in their isoelectric point on pH gradient (2-D gels)



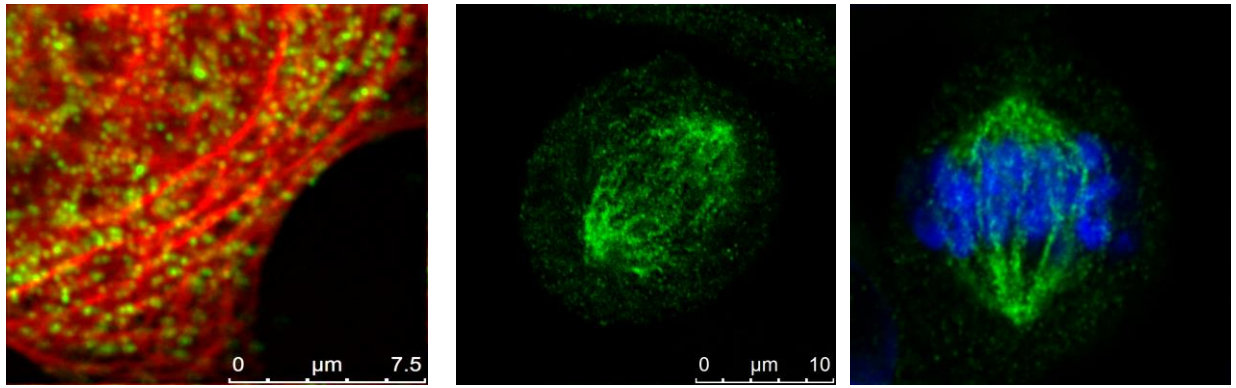
Kinesin HSET/kifC1 is implicated in the construction of microtubules in the mitotic spindle

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

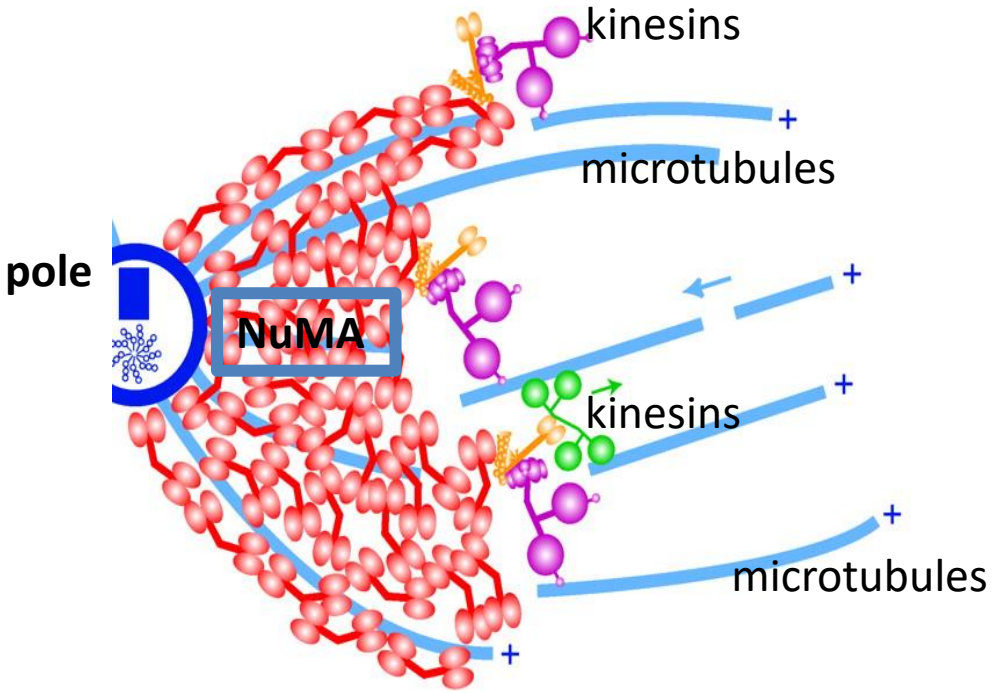
Kinesin sliding on microtubules



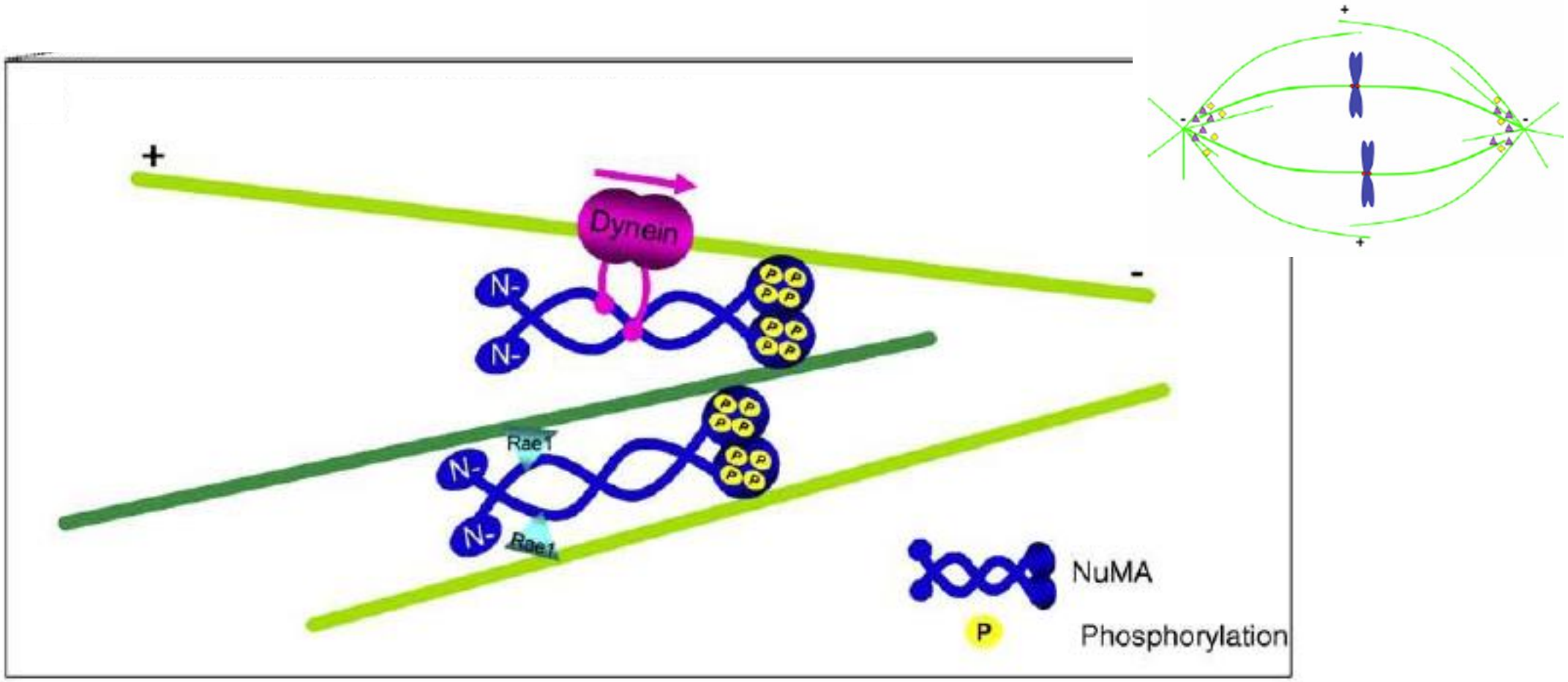
α-tubulin in the microtubules (red);
HSET/kinesinKifC1/Kif14 (green)
chromosomes (blue)



NuMA clustering in the spindle poles

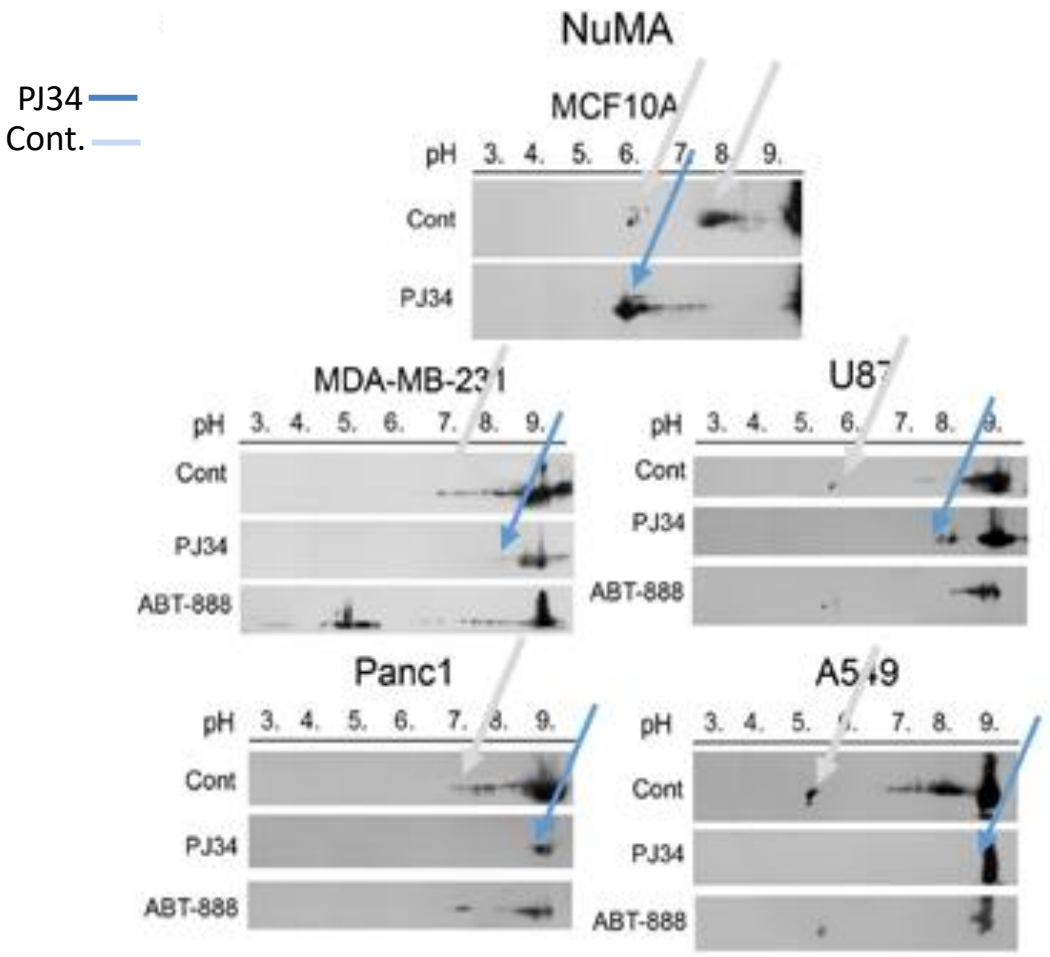


NuMA binding to proteins is crucial for its indispensable function in the mitotic spindle

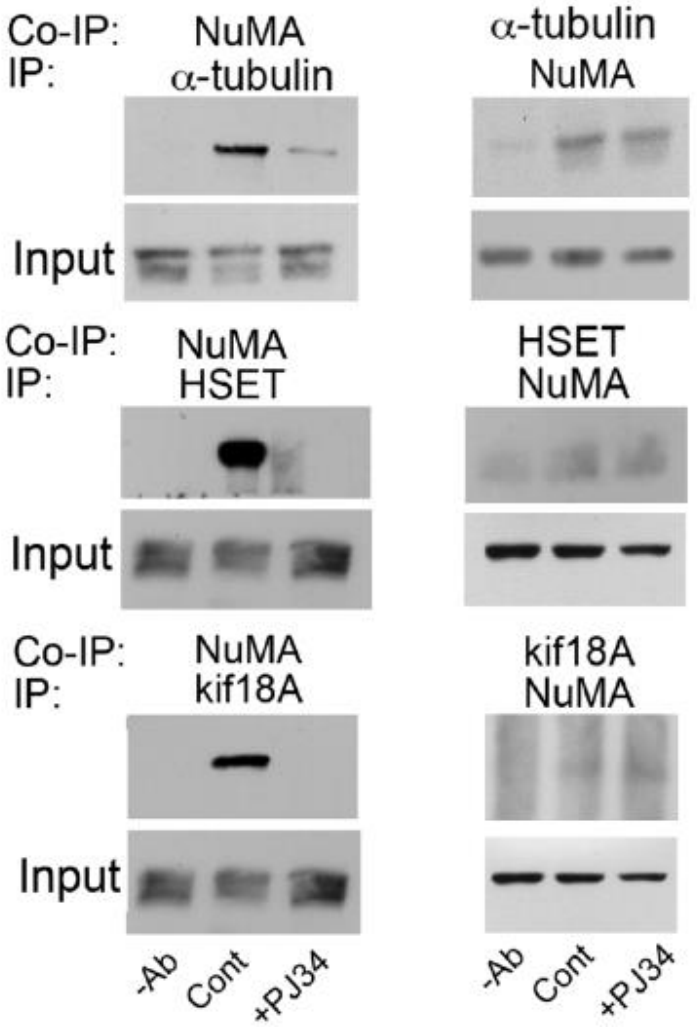


From: Radulescu and Cleveland Trends Cell Biol., 2010, 20: 214-222

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

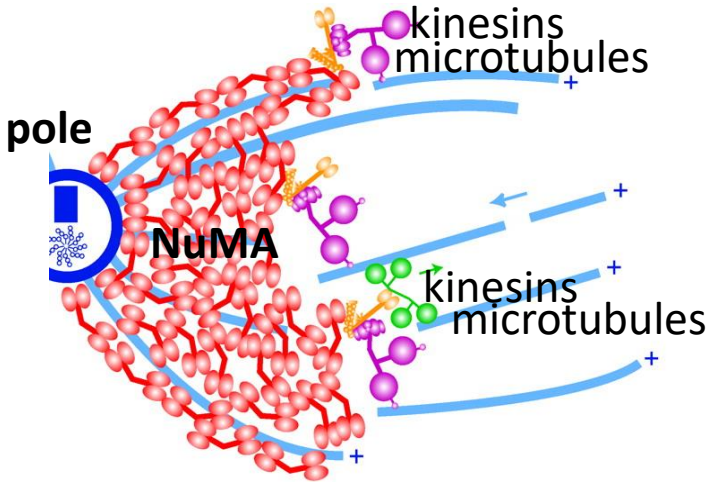


Treatment with PJ34 exclusively prevents the co-immuno-precipitation of NuMA with proteins in cancer cells



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

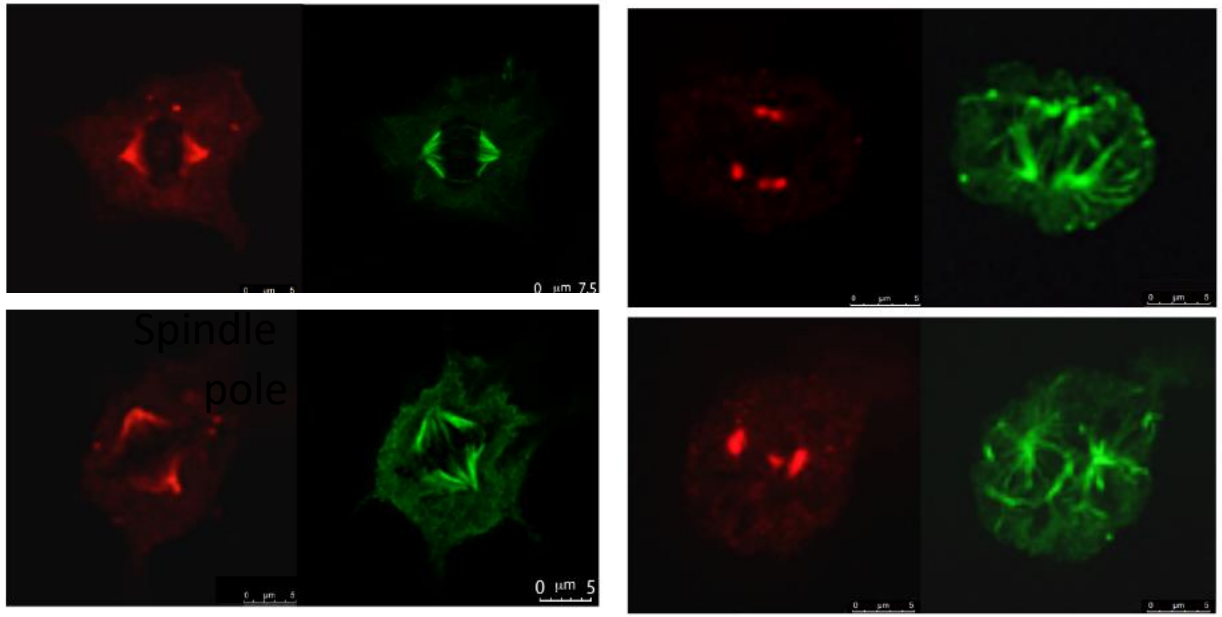
Aberrant spindles with un-clustered NuMA in the spindle poles in human cancer cells treated with PJ34



Treatment with PJ34

Healthy breast epithelial cells — MCF10A

Breast malignant epithelial cell — MDA-MB-231



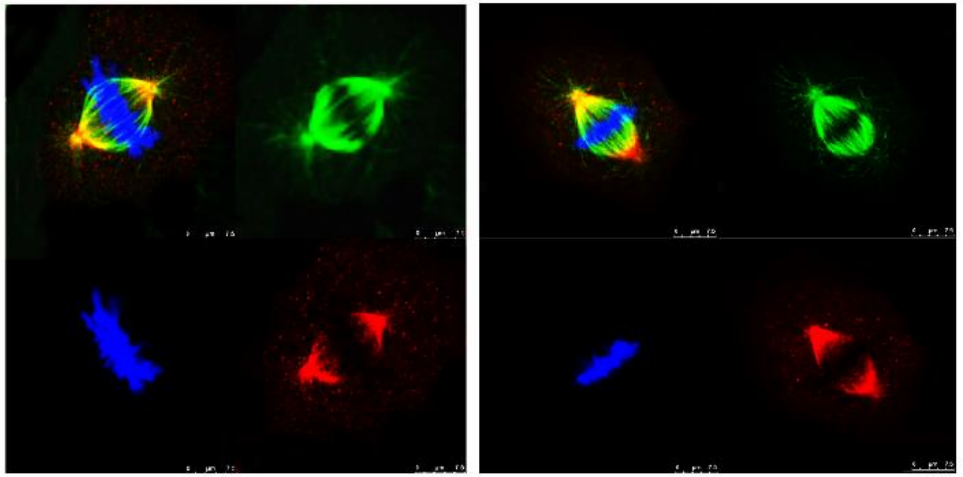
α-tubulin (microtubules)
NuMA

Visochek et al., Oncotarget, 2017

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

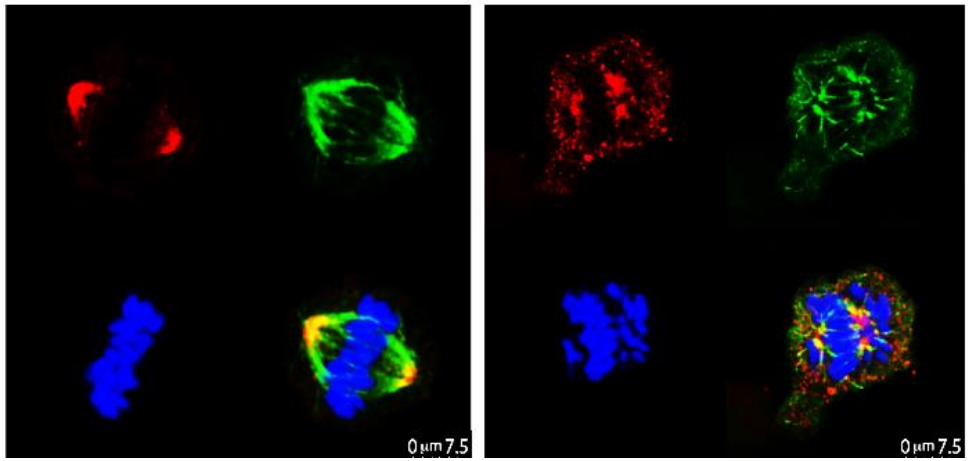
MCF10A

Control PJ34



MDA-MB-231

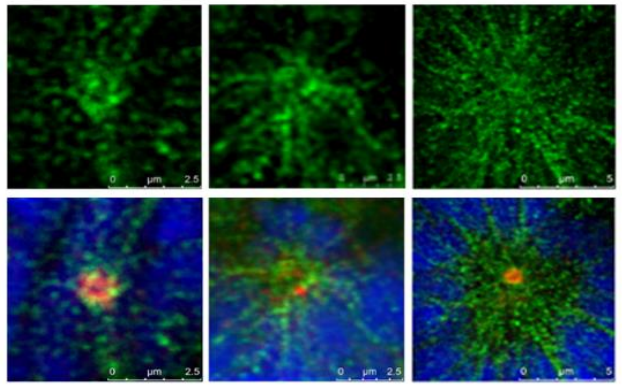
Control PJ34



α-tubulin (microtubules)
Chromosomes
NuMA

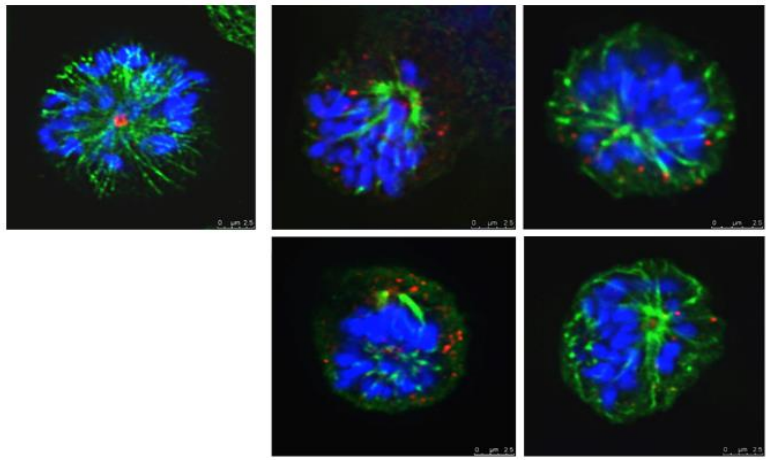
MCF10A

Control PJ34



MDA-MB-231

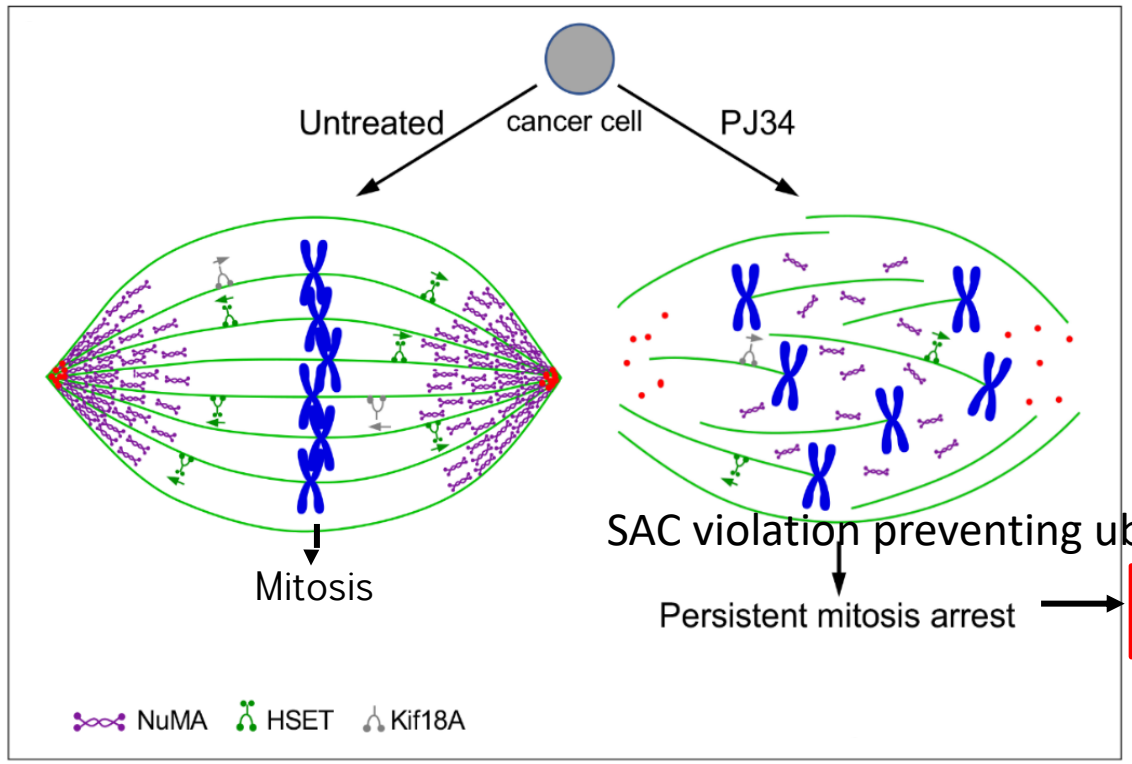
Control PJ34

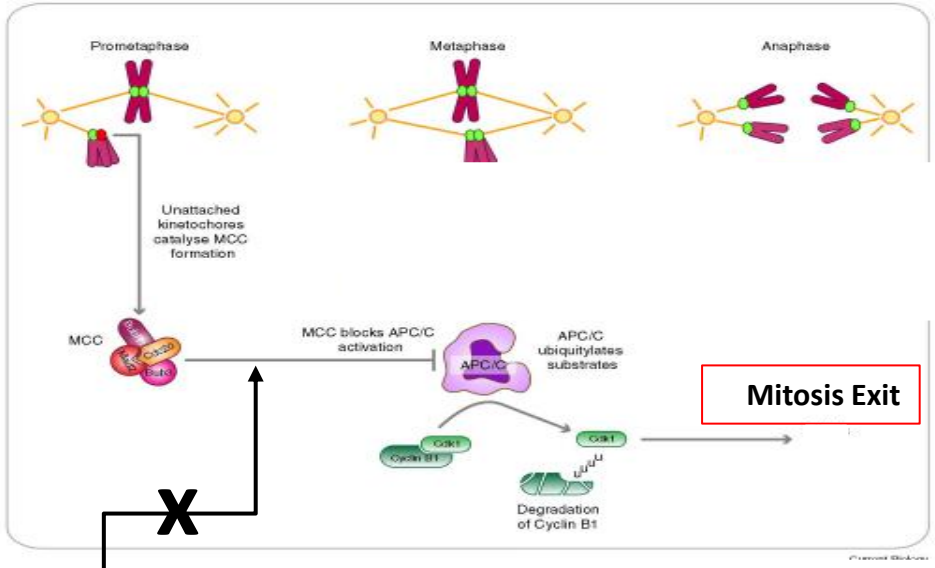


kinesinC1/HSET (microtubules)
Chromosomes
γ-tubulin (Centrosomes)

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.

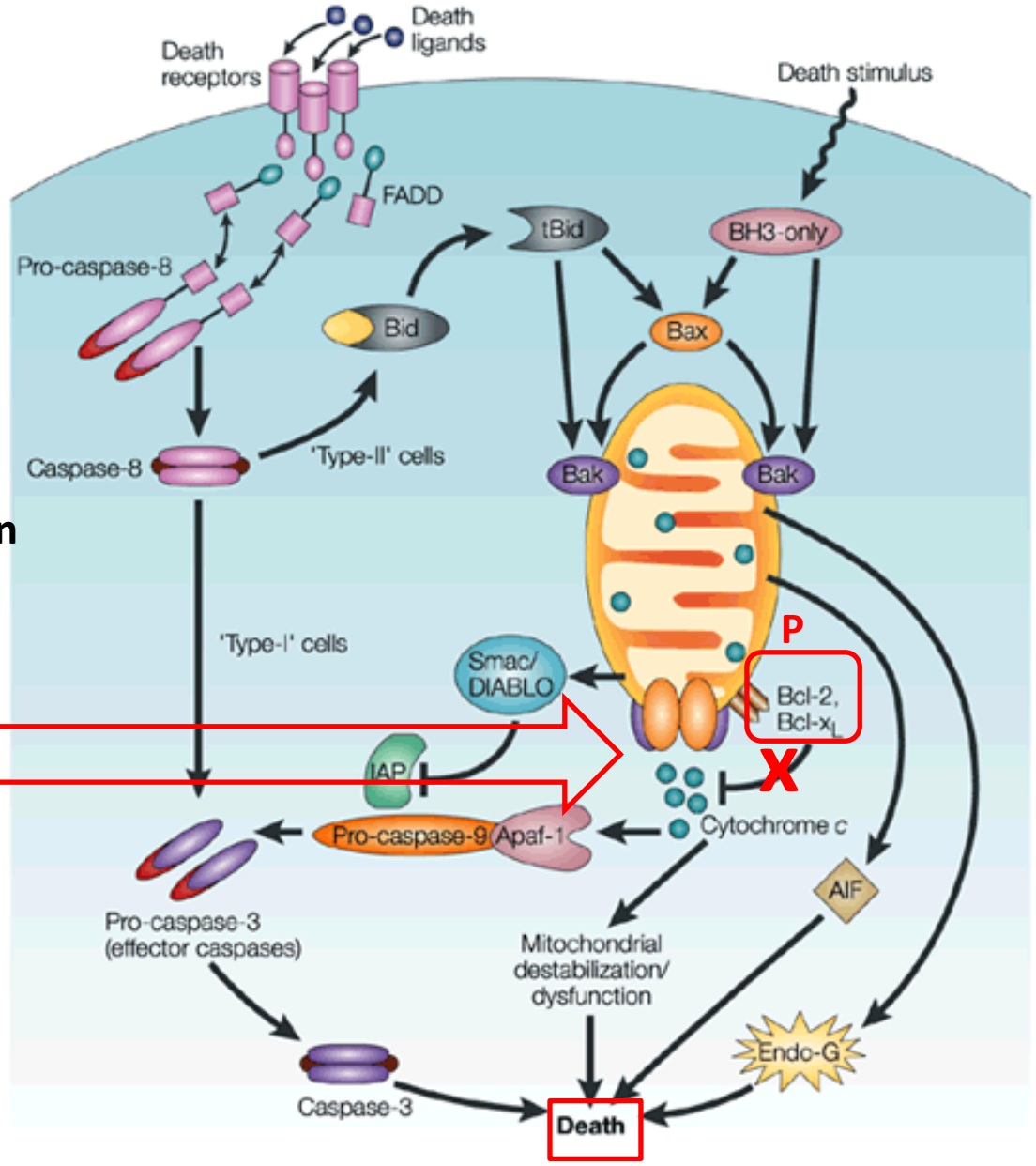
SAC violation preventing ubiquitination of cyclinB1

Persistent CyclinB1 activation of CDK

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

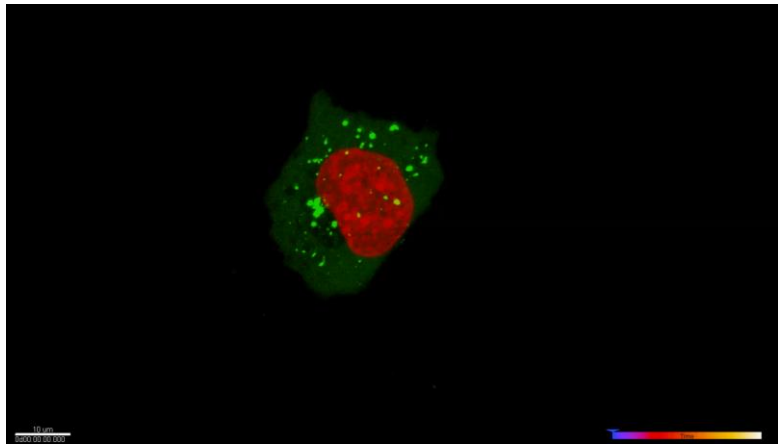
Mitosis arrest leads to
Mitotic Catastrophe cell death

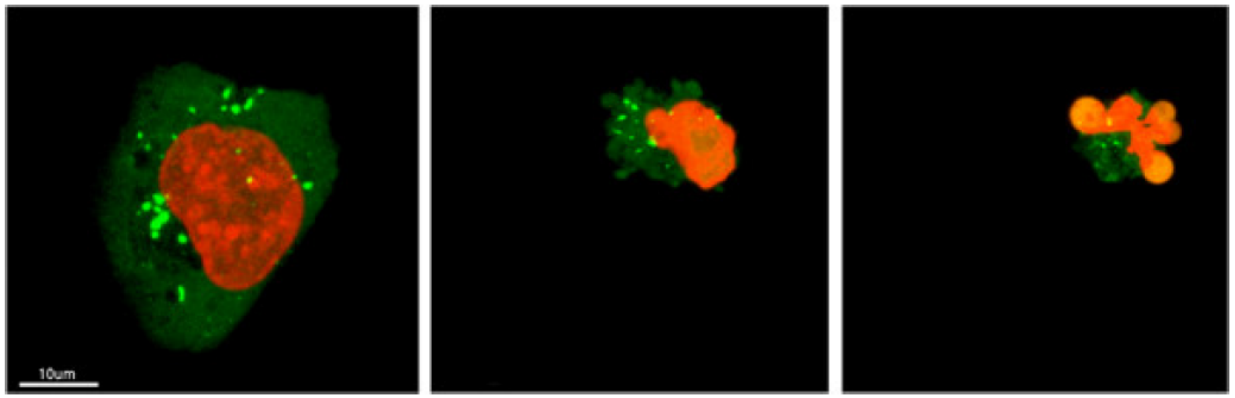
Bcl-2 and Bcl_{xL} phosphorylation by CyclinB1-activated CDK causes Cytochrome-C leakage and Cell Death



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.

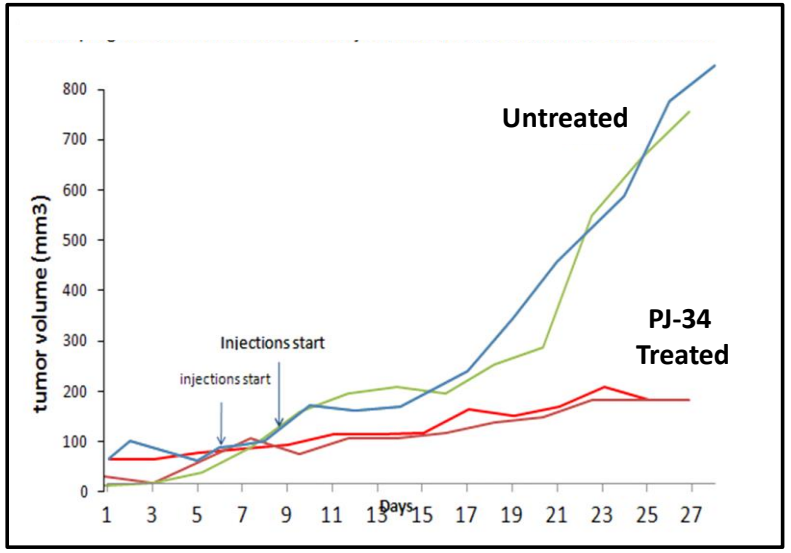




Castiel et al, JOVE, 2013

Growth Arrest of Human TN Breast Cancer Tumors MDA231 in Nude Mice Treated with PJ34

Nude mice implanted with human breast cancer cells MDA-231 were treated for 14 days with PJ34 (50 mg/kg injected i.p. every second day).



Experiments performed in collaboration with Prof Peretz and Prof Elkin in the Oncology Institute, Hadassah, Jerusalem

30 days after subcutaneous injection of cancer cells

PJ34 treated

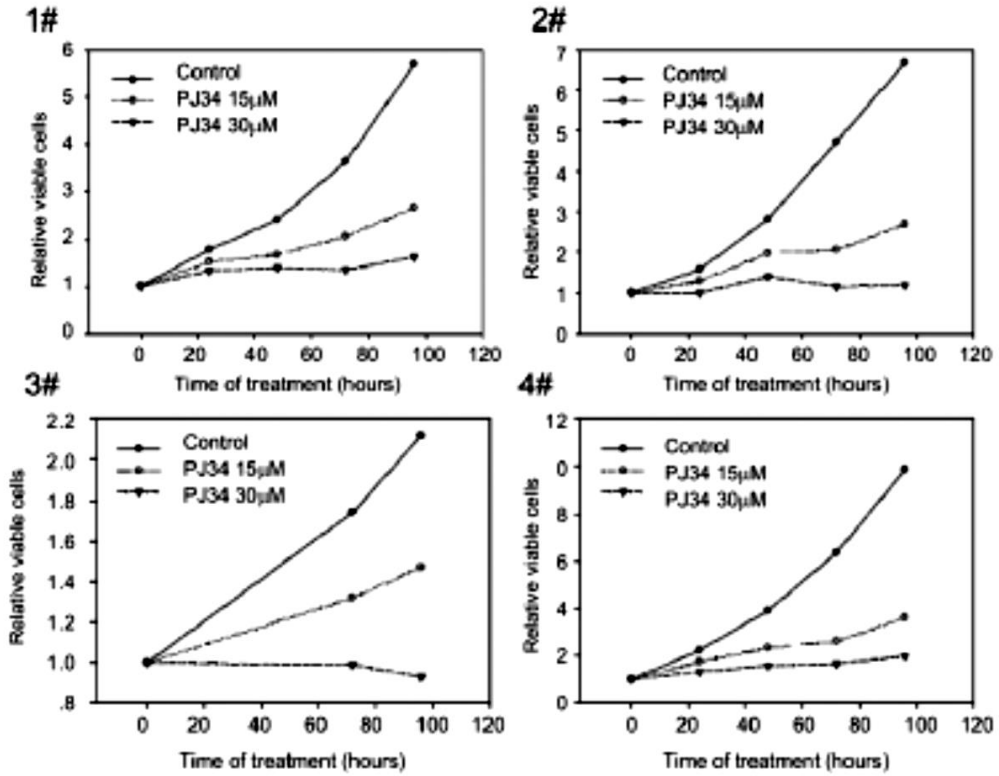
Untreated

Repeated in Pharmaseed CRO, Israel

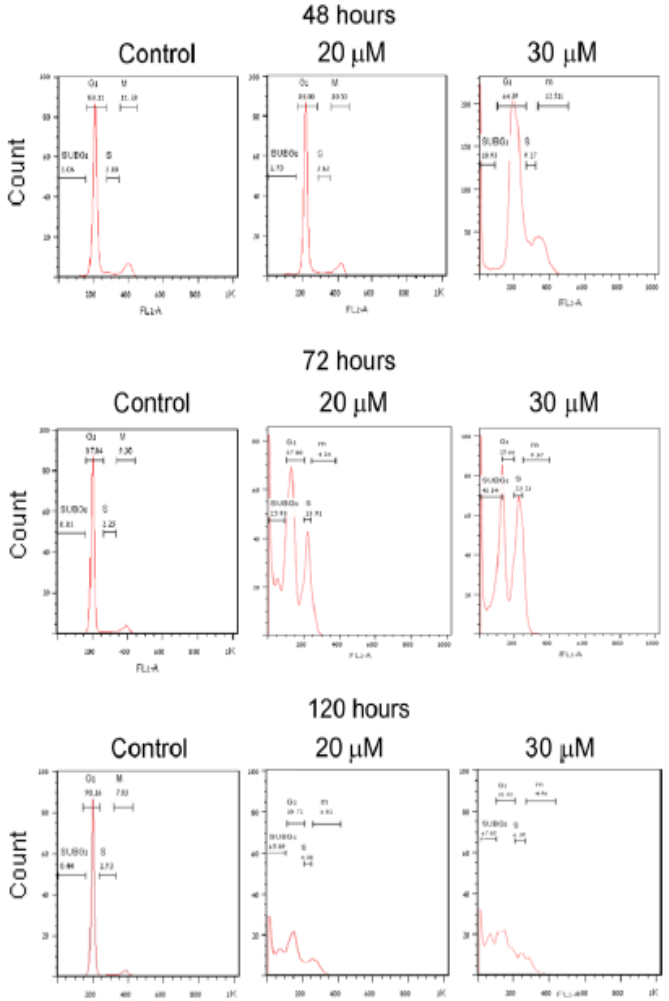
Visochek et al., https://doi.org/10.18632/oncotarget.15343

The effects of PJ34 on PANC1 human pancreas cancer cells

PJ34 cytotoxicity in cell culture prepared from patients-derived xenografts. Cell cultures derived from four different types of pancreas cancer xenografts were incubated with PJ34 at the indicated concentrations. Cell survival was quantified after the indicated incubation period with PJ34.



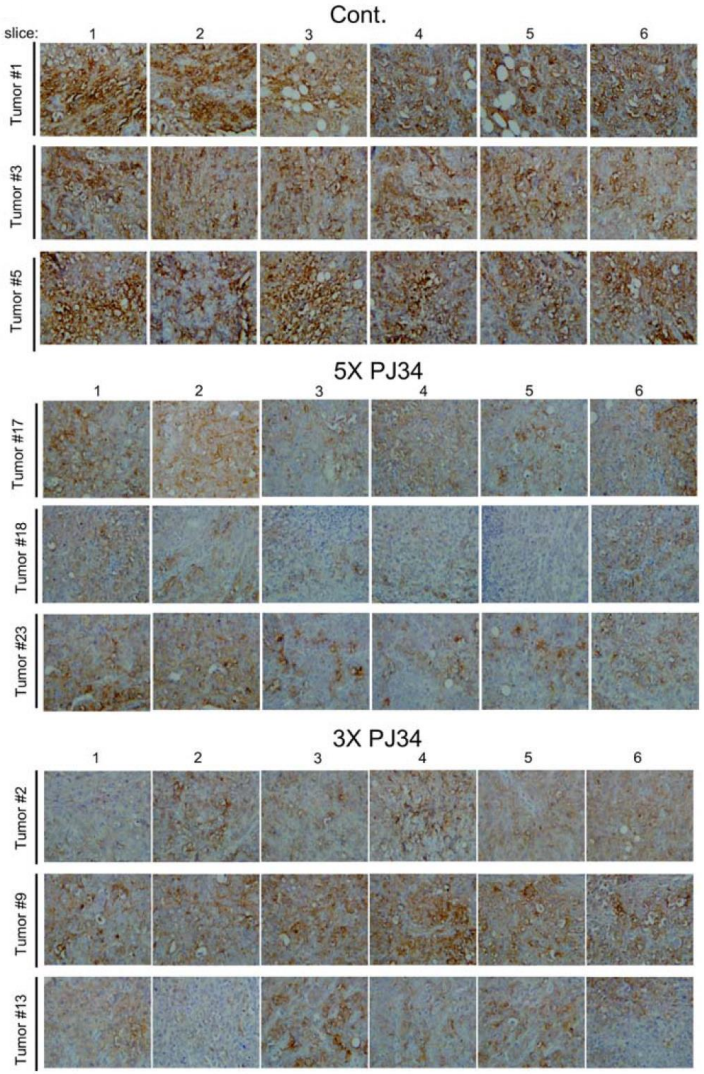
PJ34 causes Mitosis Arrest and Cell Death measured by flow-cytometry in human pancreas PANC1 cancer cells



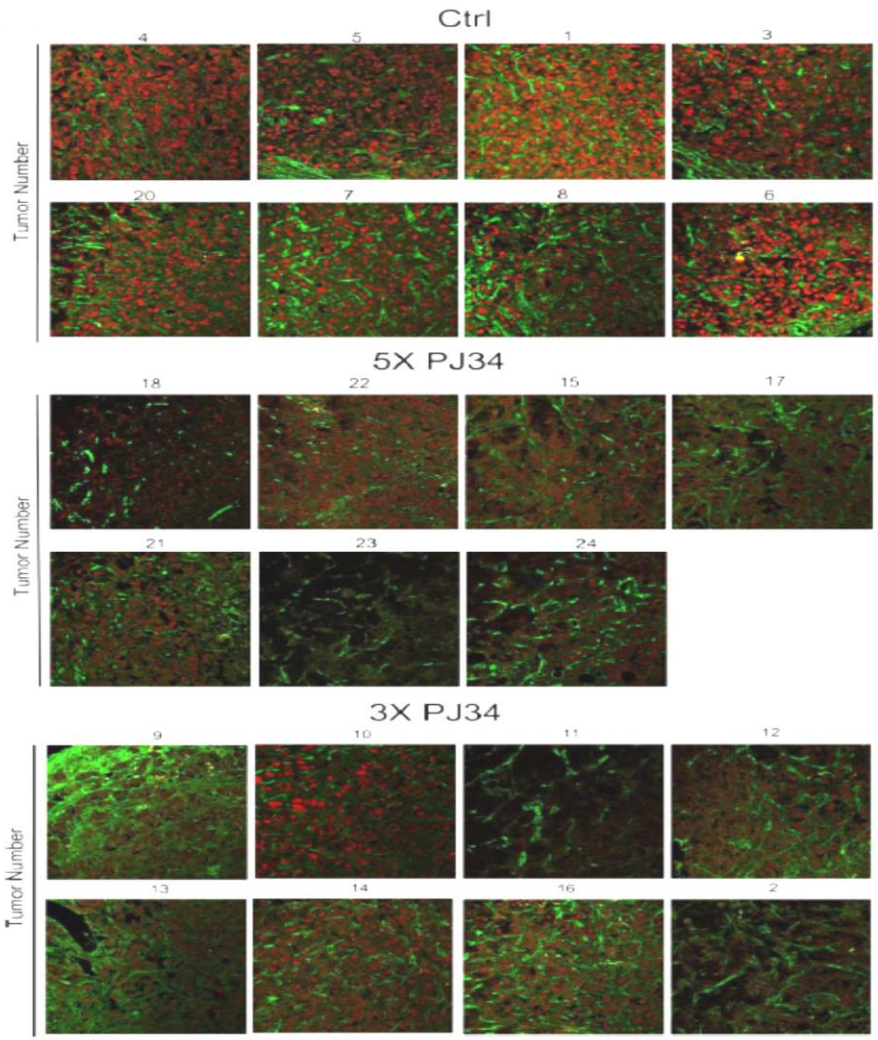
Visochek et al., Oncotarget, 2019

Human PANC1 cells eradication in tumors that developed in nude mice measured by immunohistochemistry (A. ALA labeling; B. Ku-80 labeling (red)) in slices prepared from excised tumors, 30 days after 14 days daily treatment (IV) with PJ34. Fibroblasts (green) in the tumors were not impaired.

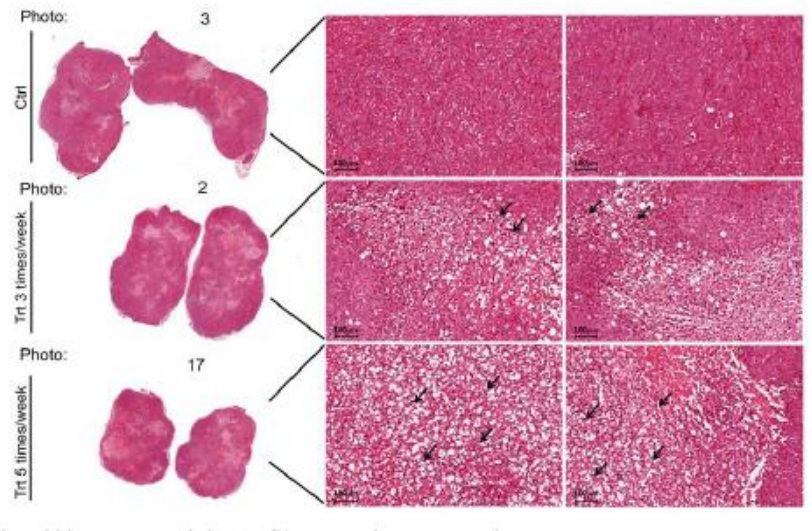
A



B

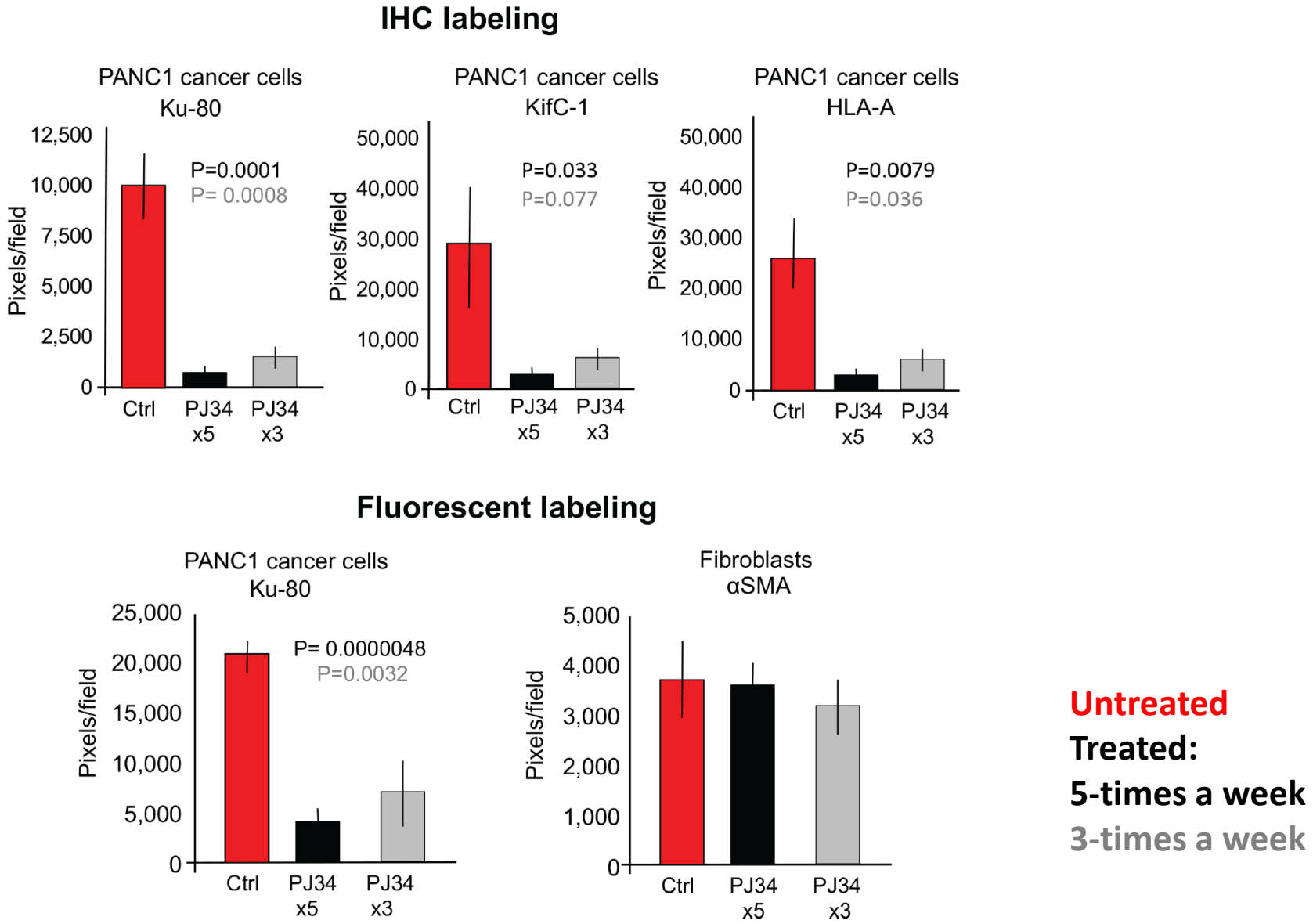


Cell death in Haemotoxylin and Eosin stained slices of PANC1 tumors



Visochek et al., Oncotarget, 2019

A quantitative presentation of PANC1 cells eradication measured in slices of tumors developed in nude mice by immunohistochemistry 30 days after 14 days daily IV treatment with PJ34 (50 mg/Kg)



Untreated

Treated:

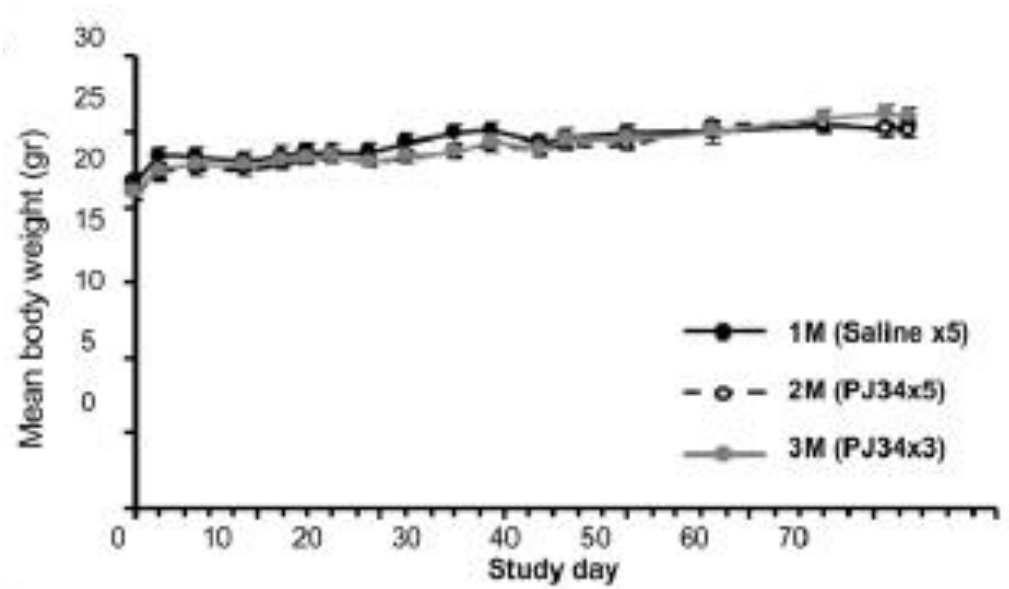
5-times a week

3-times a week

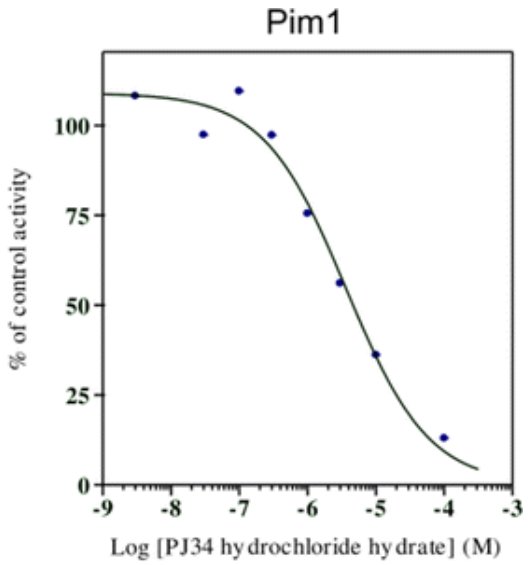
Visochek et al., Oncotarget 2019

The effect of PJ34 in xenografts developing PANC1 tumors

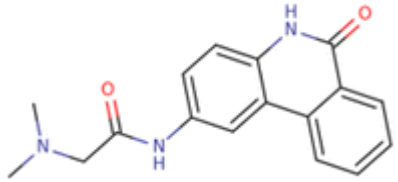
Treatment with PJ34 did not impair the weight gain of nude mice developing PANC1 tumors. PJ34 was injected IV (60 mg/Kg dissolved in 100 µl saline, approximately 1 mg PJ34 per mouse). Control nude mice were injected daily with saline.



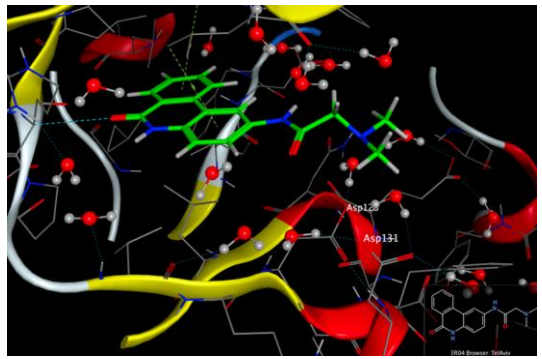
PJ34 inhibits the activity of the kinase pim1 and of tankyrase1, both modify NuMA in human cancer cells and promote its protein-binding capacity



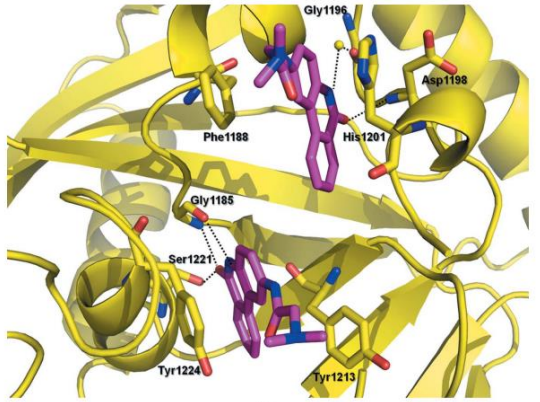
IC_{50}=3.7 μM



kinase pim1

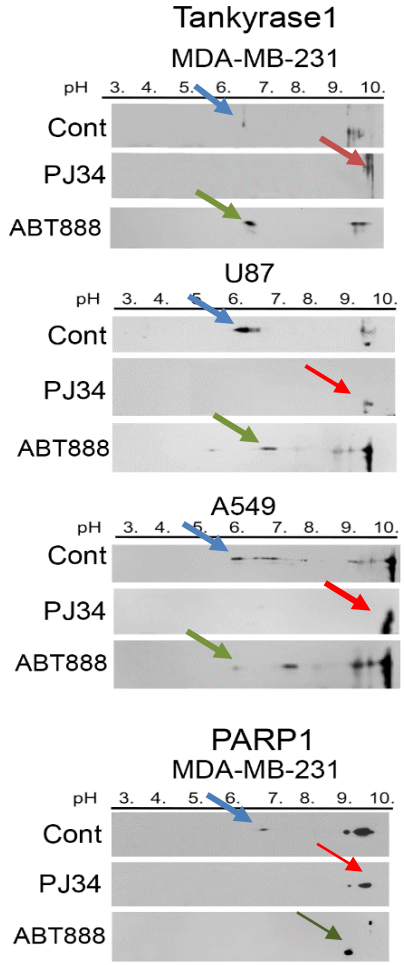


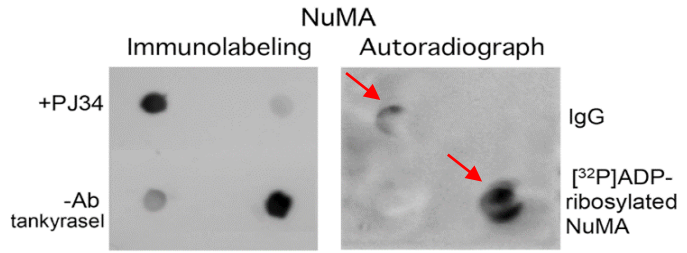
tankyrase1



IC_{50}=1. μM

Tankyrase1 and NuMA polyADP-ribosylation





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

Kirby CA., et al. Acta Cryst, 2012

Visochek et al., Oncotarget, 2017

Summary

- Preventing the post-translational modification of NuMA by inhibiting two proteins exclusively expressed in human cancer cells causes Mitotic Catastrophe cell death
- The treatment with PJ34 causes eradication of malignant cells during mitosis (at the anaphase), while healthy proliferating cells are spared.
- In this mechanism, cancer cells are eradicated by PJ34 regardless of their genetic mutations. The more rapidly they proliferate, the more rapidly they are eradicated.
- This mechanism leads to a new mode of therapy for aggressive cancers

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Thank you