Microtubule dynamic instability, implication in oncopharmacology and Modeling

Stéphane Honoré

Centre de Recherche en Oncologie Biologique et Oncopharmacologie (CRO2)

Aix-Marseille Université

Centre de
Recherche en
Oncologie biologique et
Oncopharmacologie

PHAR-MATHO-TUBULE project

Mathematical Modeling of the pharmacological effects of microtubule-targeted drugs on **microtubule dynamic instability** and cellular processes involved in cancer progression: i.e **cell migration**

Stéphane Honoré, Florence Hubert
Christophe Gomez

Ayuna Barlukova (phD)
Marie Petit (PhD)
Remi Tesson (PhD)

Poster

Diana White (post-doc)
Sarah Oddoux (post-doc)
Clément Ricard (Post-doc)

Next Talk

aviesan
alliance nationale pour les sciences de la vie et de la santé

PLAN cancer 2014-2019

A*Midex
Initiative d'excellence Aix-Marseille

INVESTISSEMENTS D'AVENIR

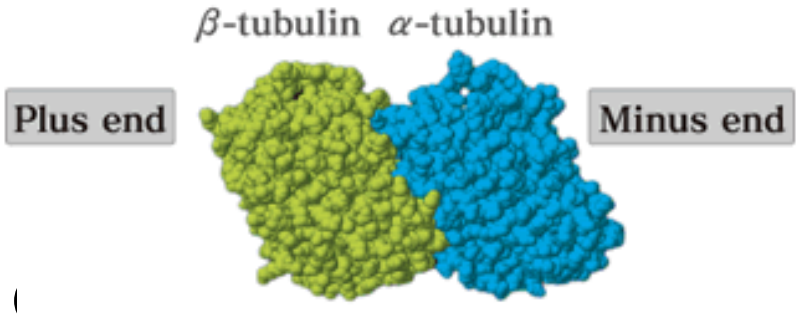
Molecular apects of Microtubule Dynamic Instability

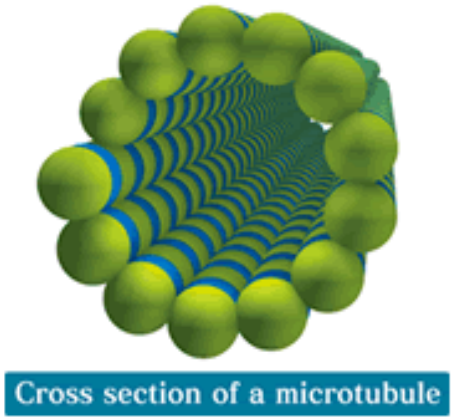
Tubulin polymerization

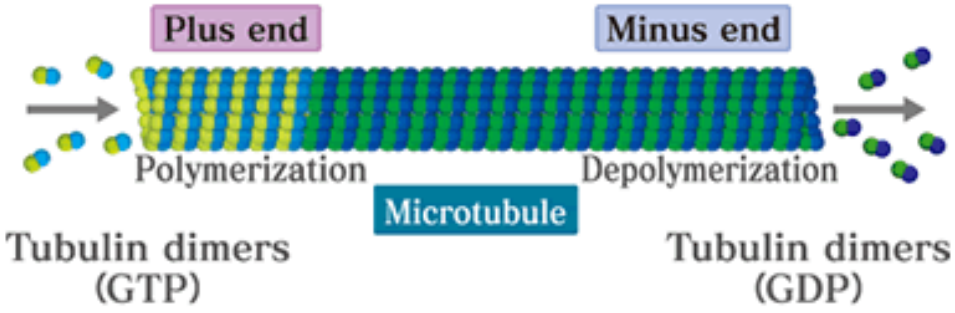
- α-tubuline lie le
- β-tubuline lie le
en GDP+ Pi

•L'α et la β tubulir
dimères

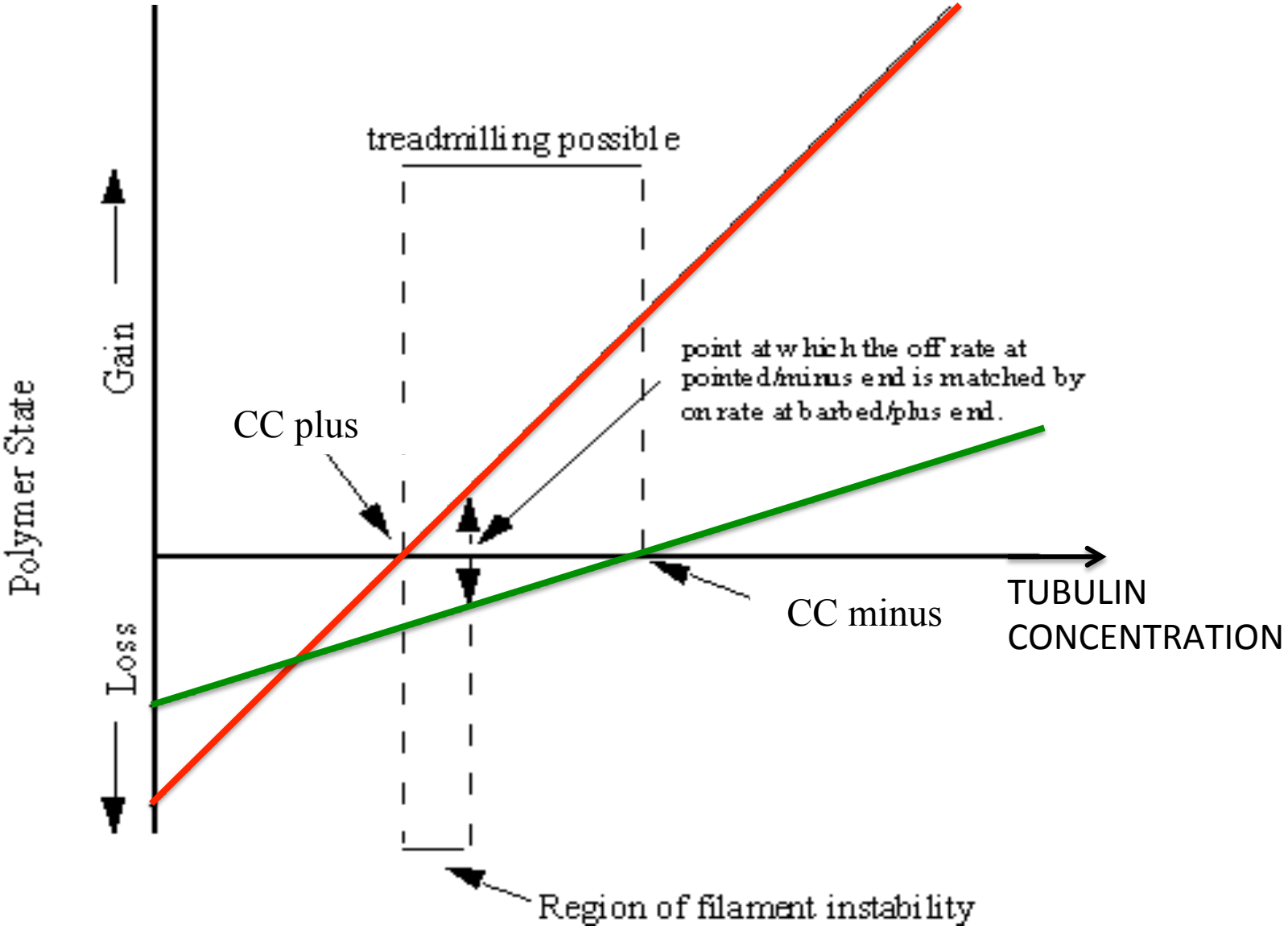
•-> Induit une pola
microtubules





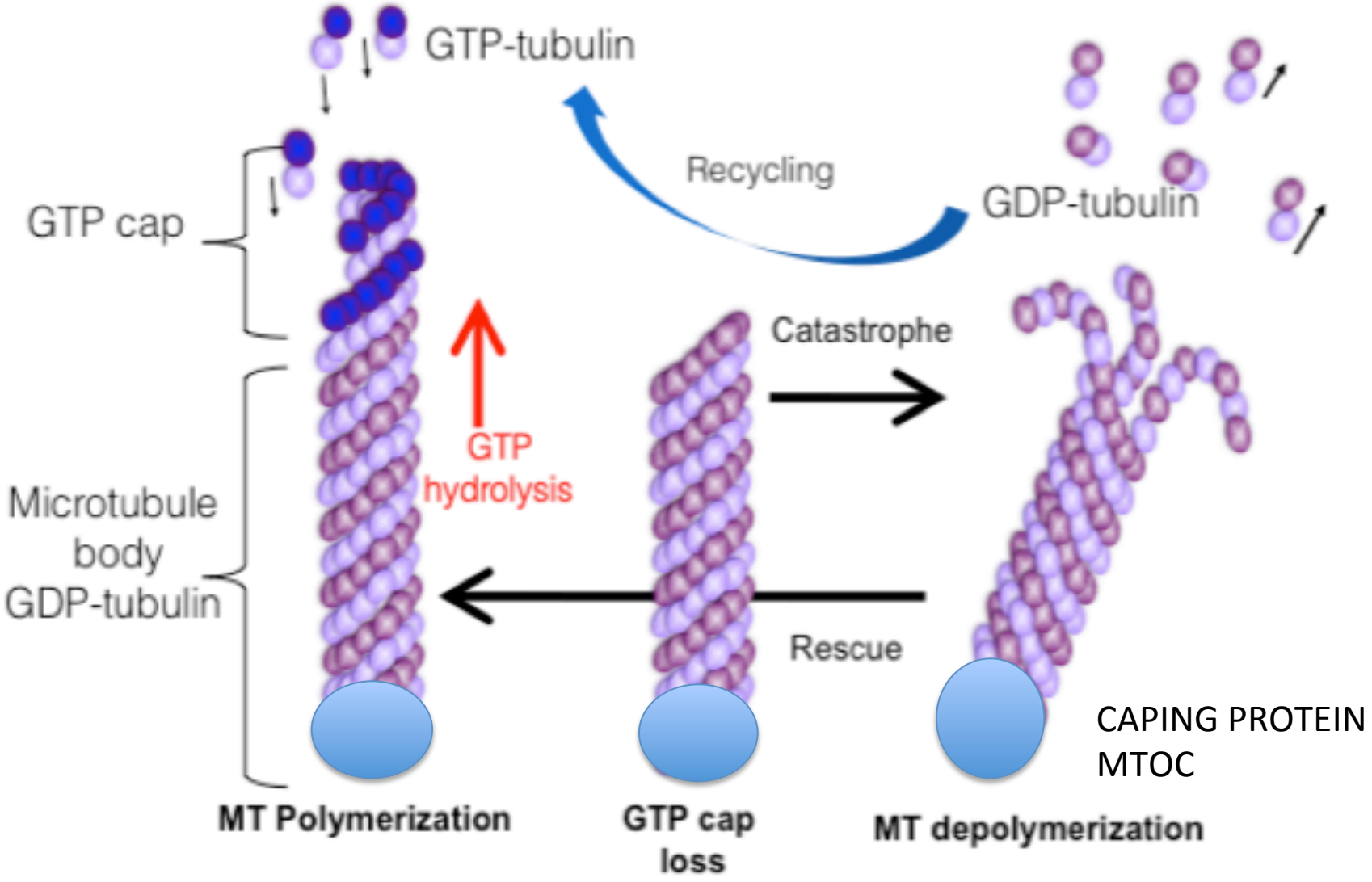


MT polymerization depend on tubulin critical concentration

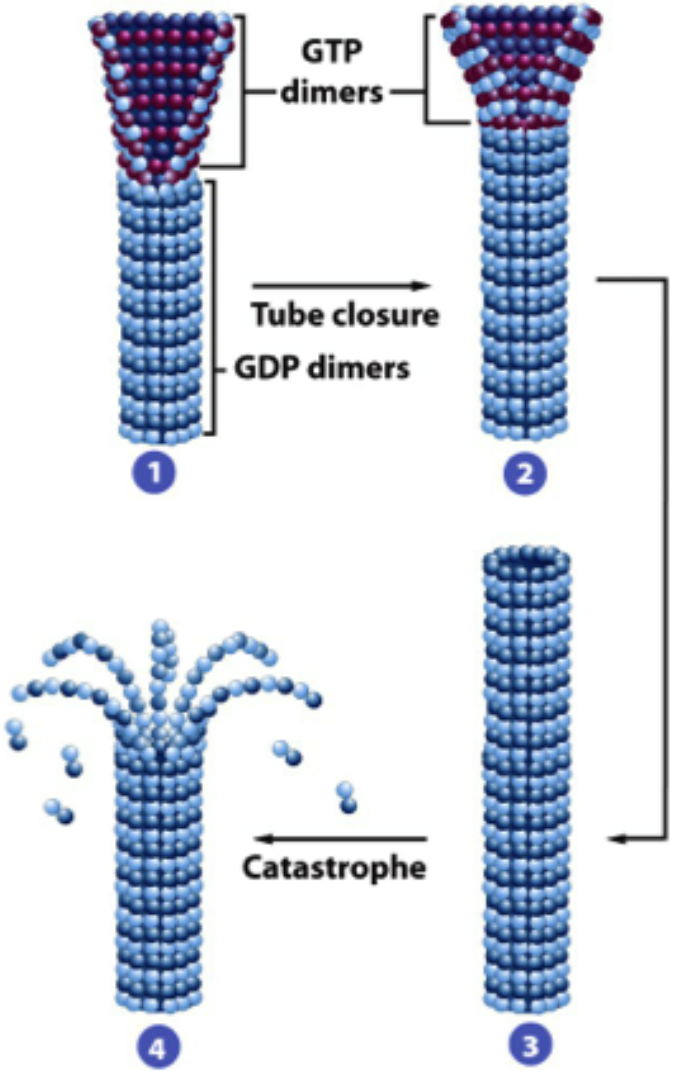


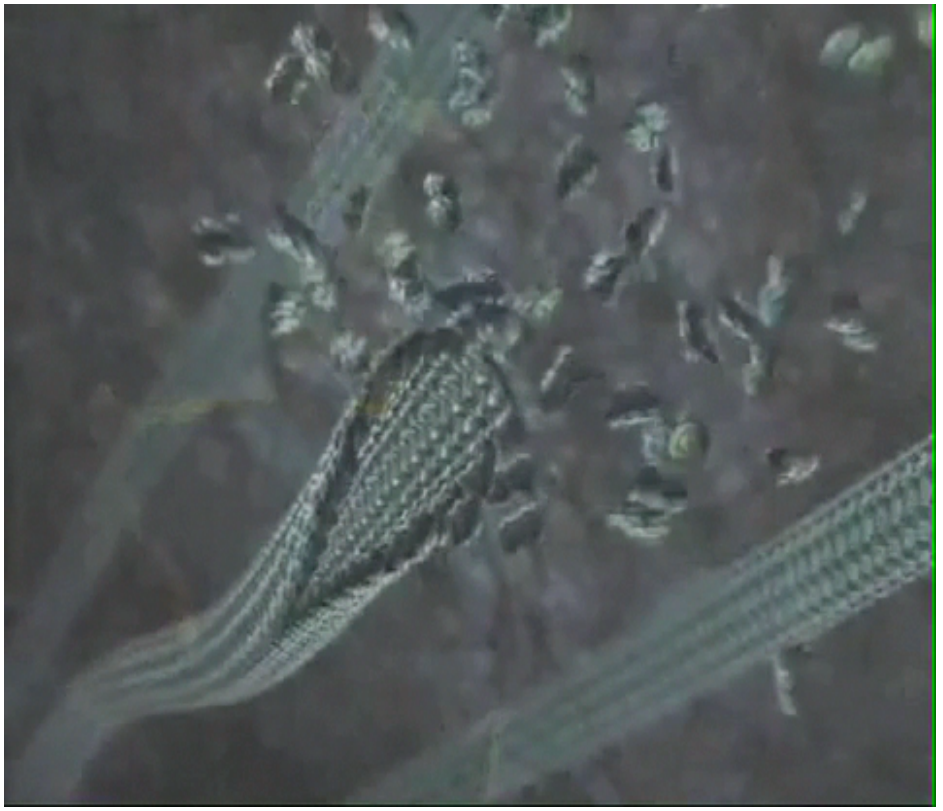
Microtubule dynamic instability

GTP hydrolysis and GTP cap

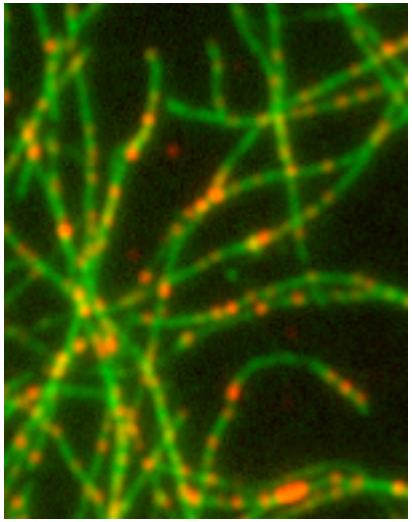


« GTP hydrolysis and Catastrophe »

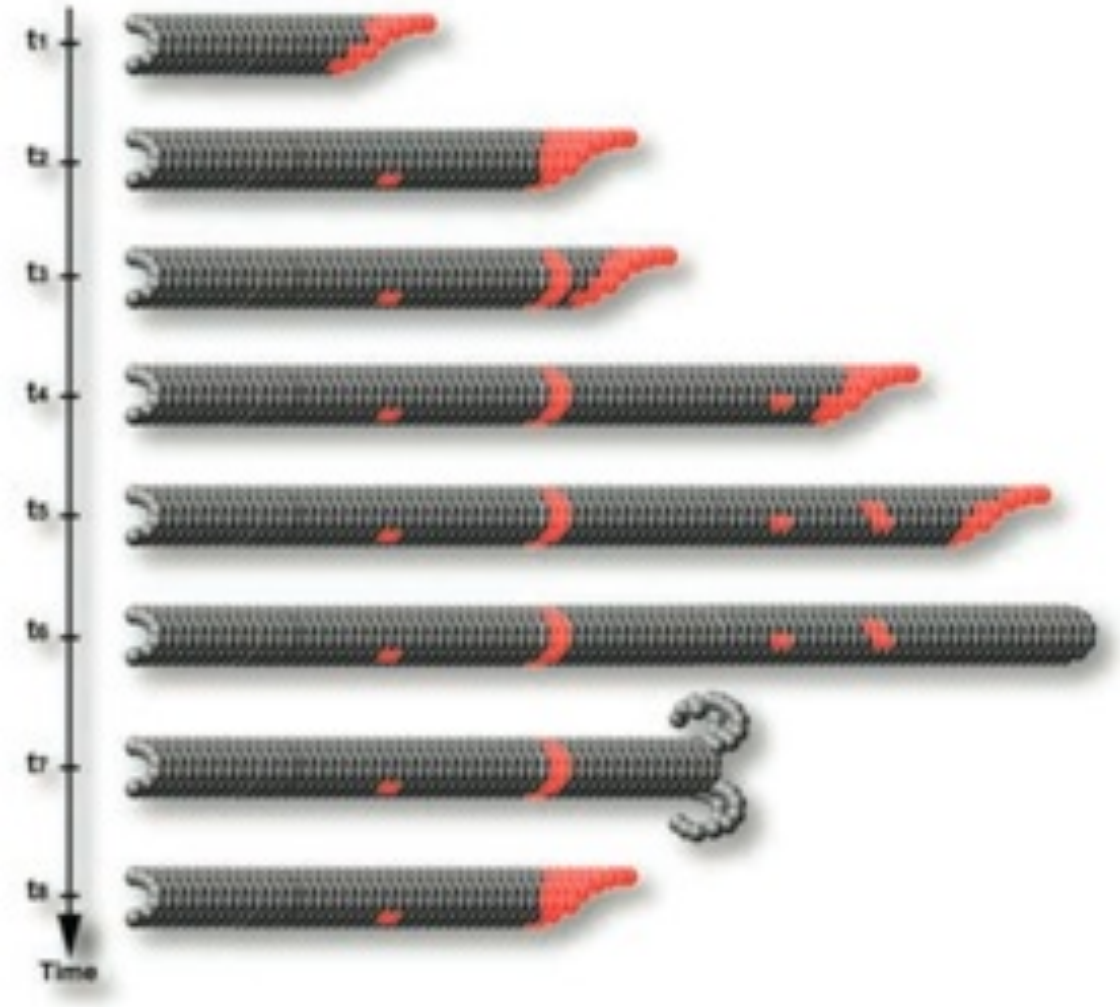




« GTP-Remnant and Rescue »



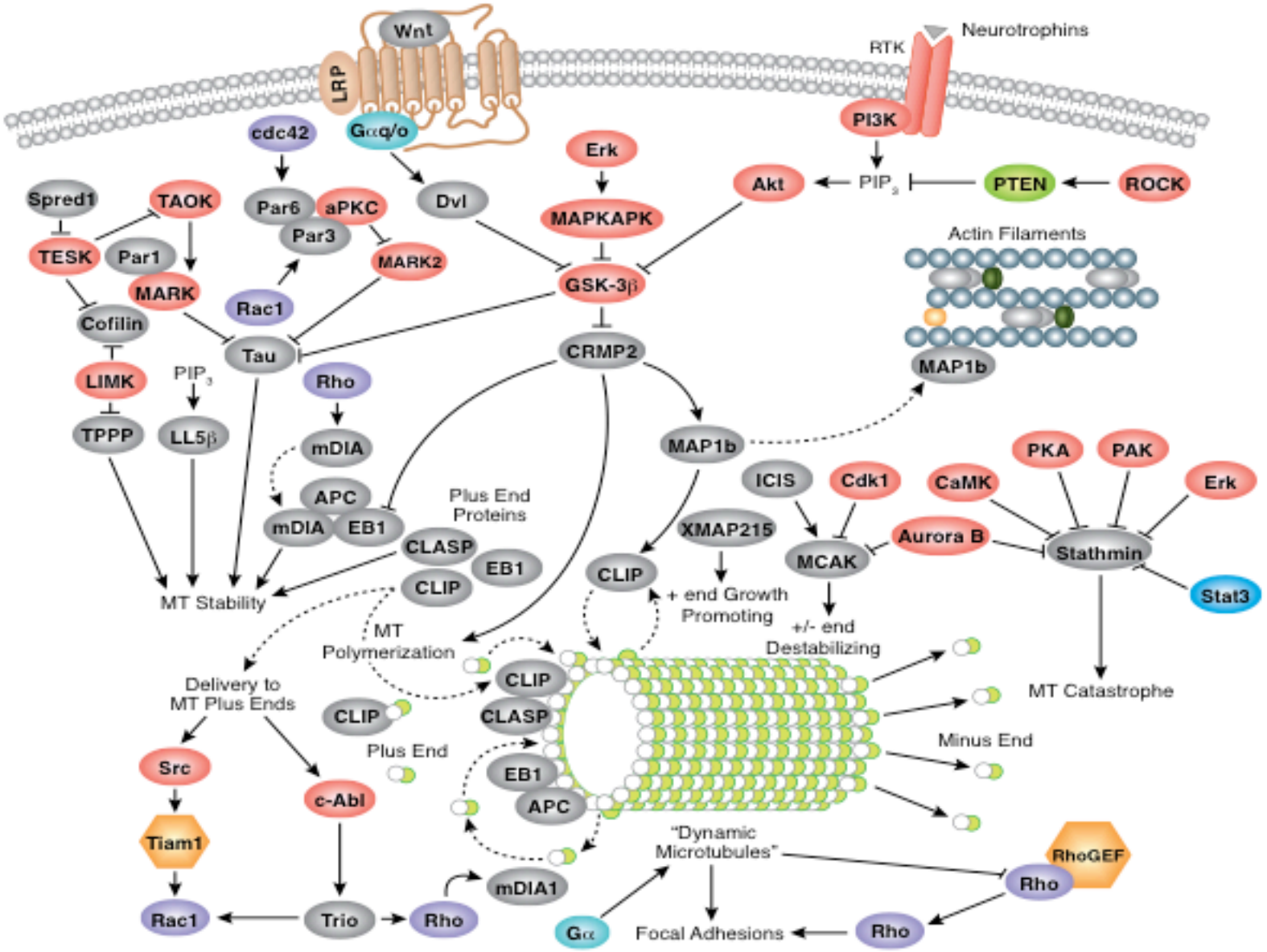
hMB11
Anti-GTP tubulin
(F Perez)



F Perez lab

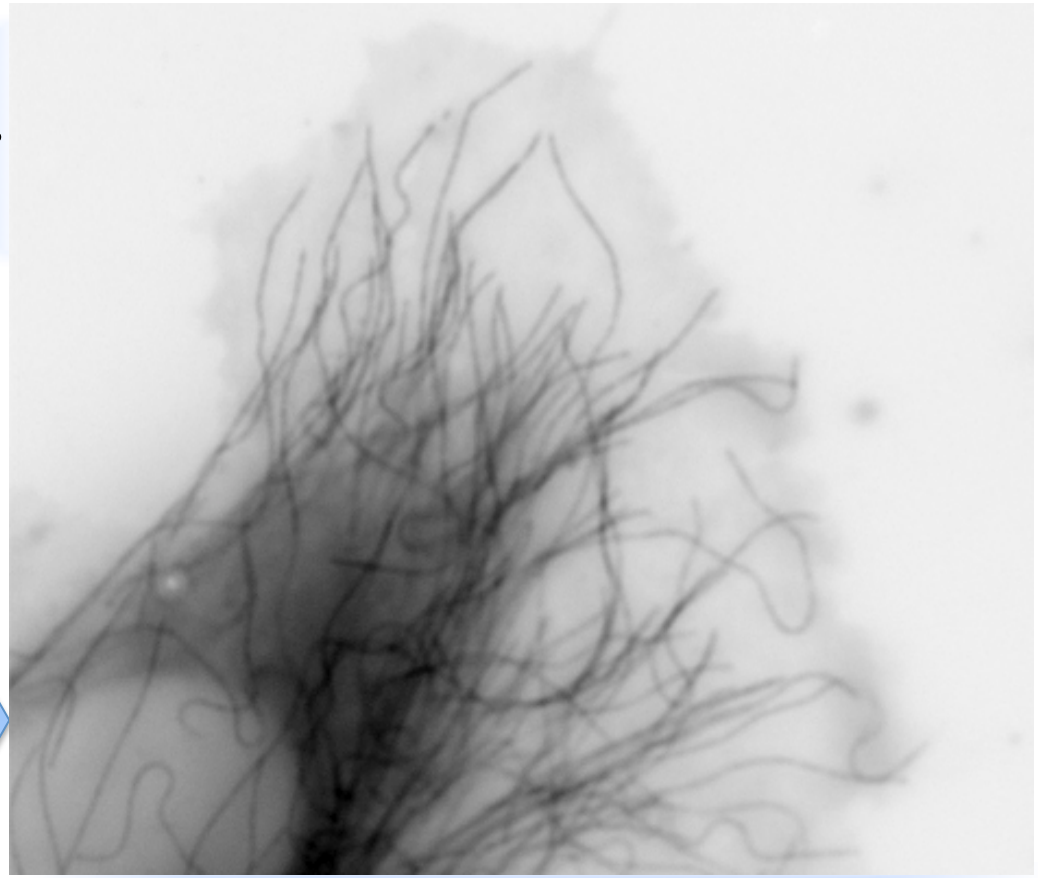
MT dynamic instability in living cells

Microtubule Dynamics regulation in cells



Integration and signal transduction

Extra and intra cellular Stimuli



Tumor, endothelial, immune cells

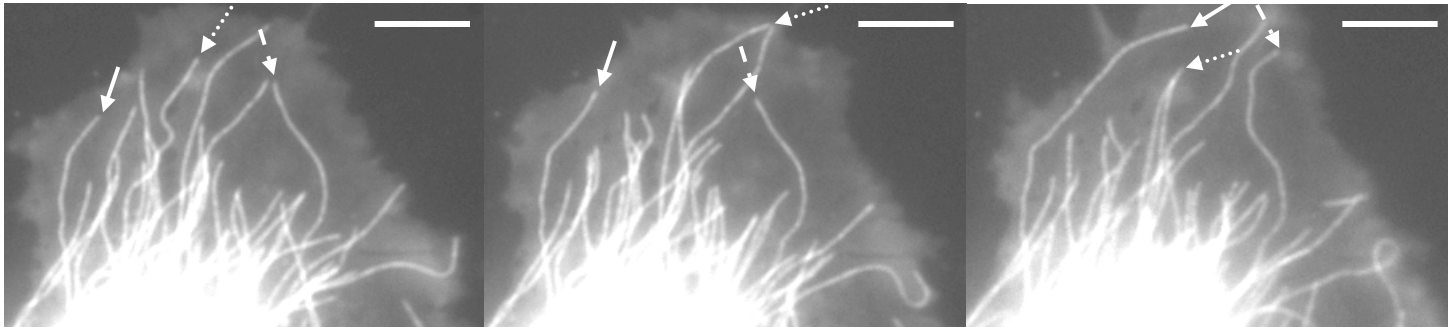
Polarity
Shape
Adhesion
Transport

Immunological synapse

Migration
Differentiation
Mitosis

TUMOR PROGRESSION

Measuring MT dynamic instability



0 s **4 s** **8 s**

Growth rate, length, duration

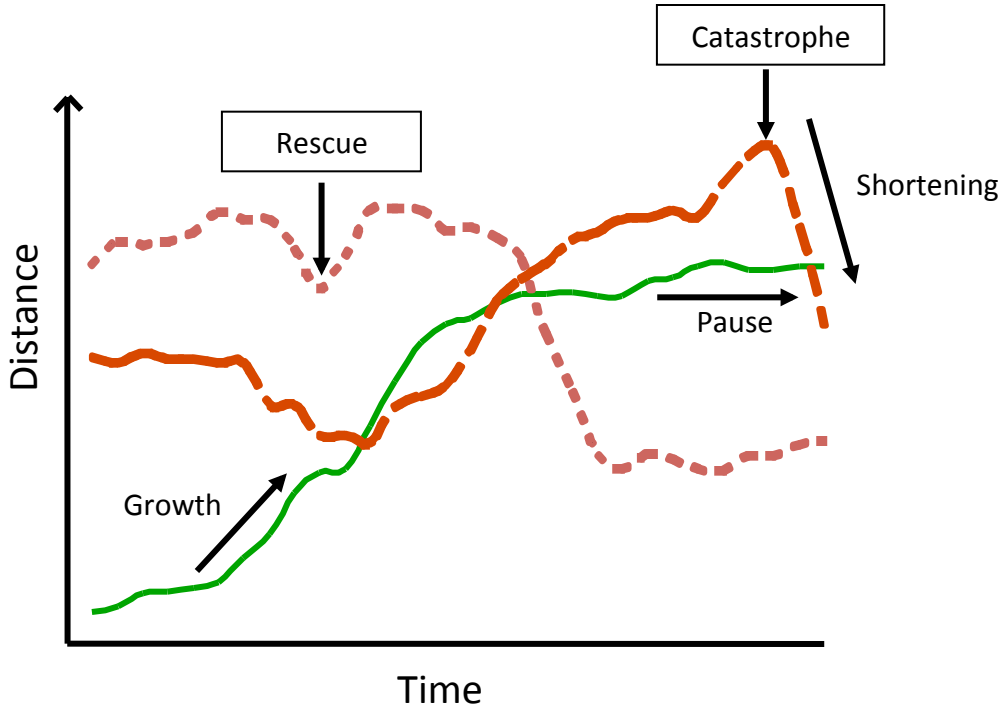
Shortening rate, length, duration

Pause duration

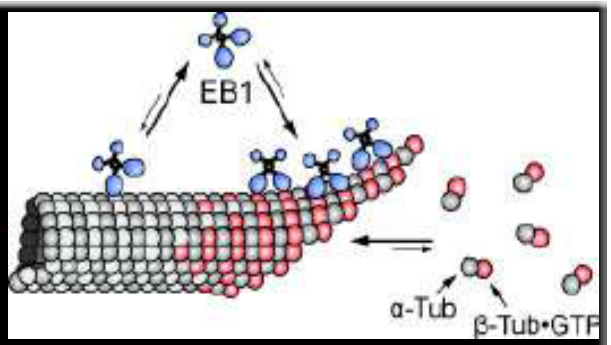
Transition frequencies (both time and spatial)
(catastrophe, rescue)

Time spent in growing,
shortening and Paused states

Overall Dynamicity



Microtubules / EBs system in Living Cells



EB2-EB3-EB1

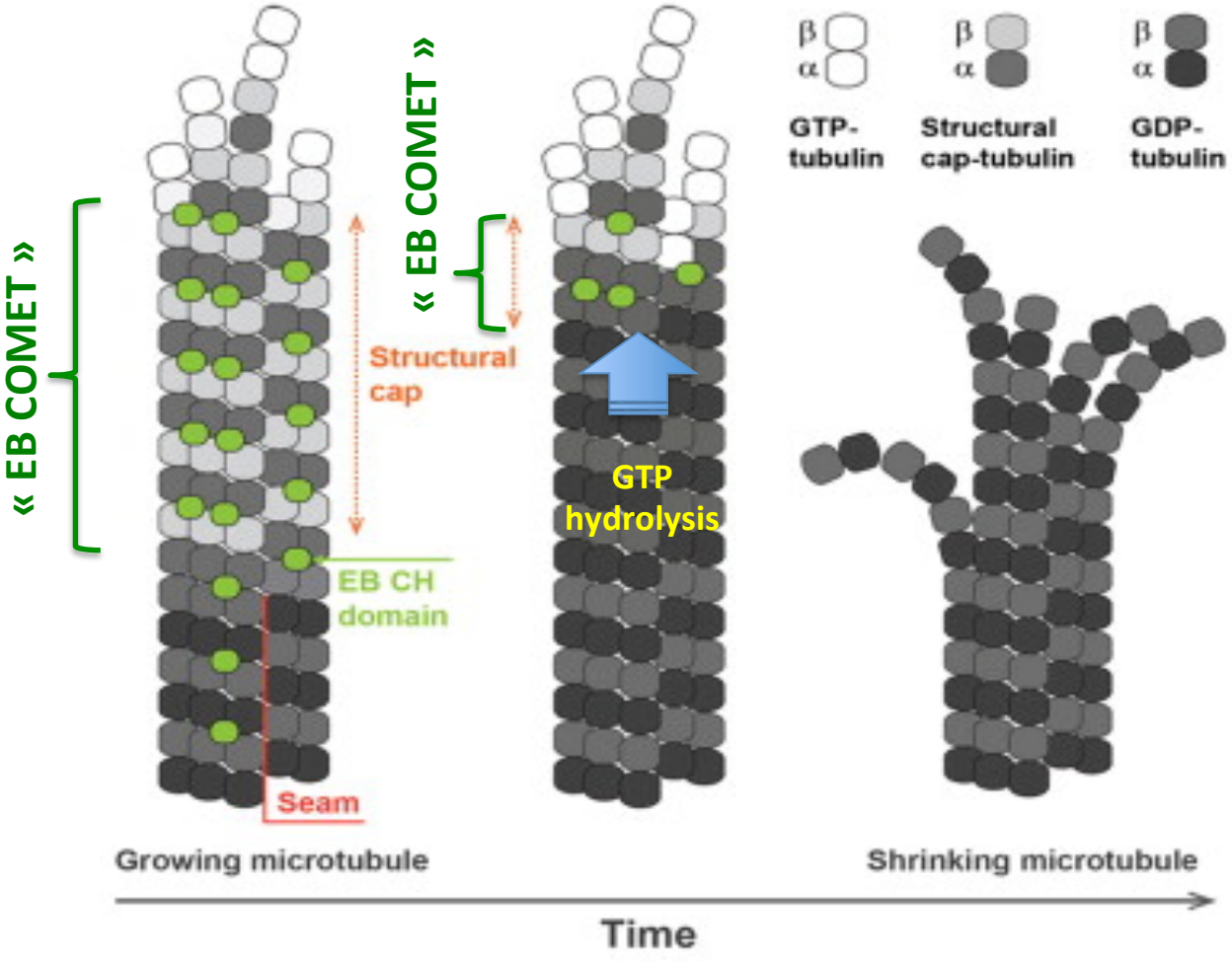
EBs comets ≈ GTP/GDP-Pi stabilizing cap

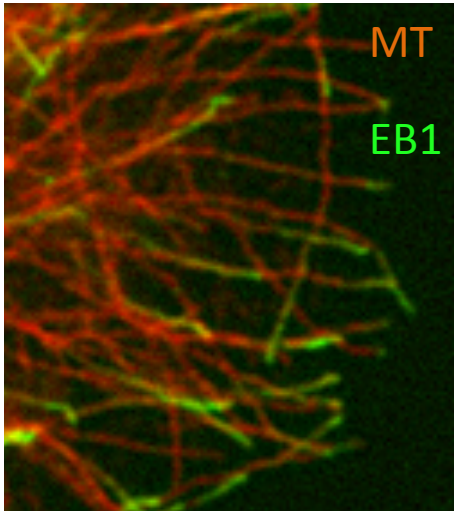
- highly dynamic and tightly regulated
- Important for intracellular transport, mitosis, migration, structural support, traffic of proteins and cellular components, energetics and metabolism

Glioblastoma U87 cell

End-Binding proteins bind to and regulate the structural MT « stabilizing » cap

Adapted from Maurer et al., 2012 and 2014

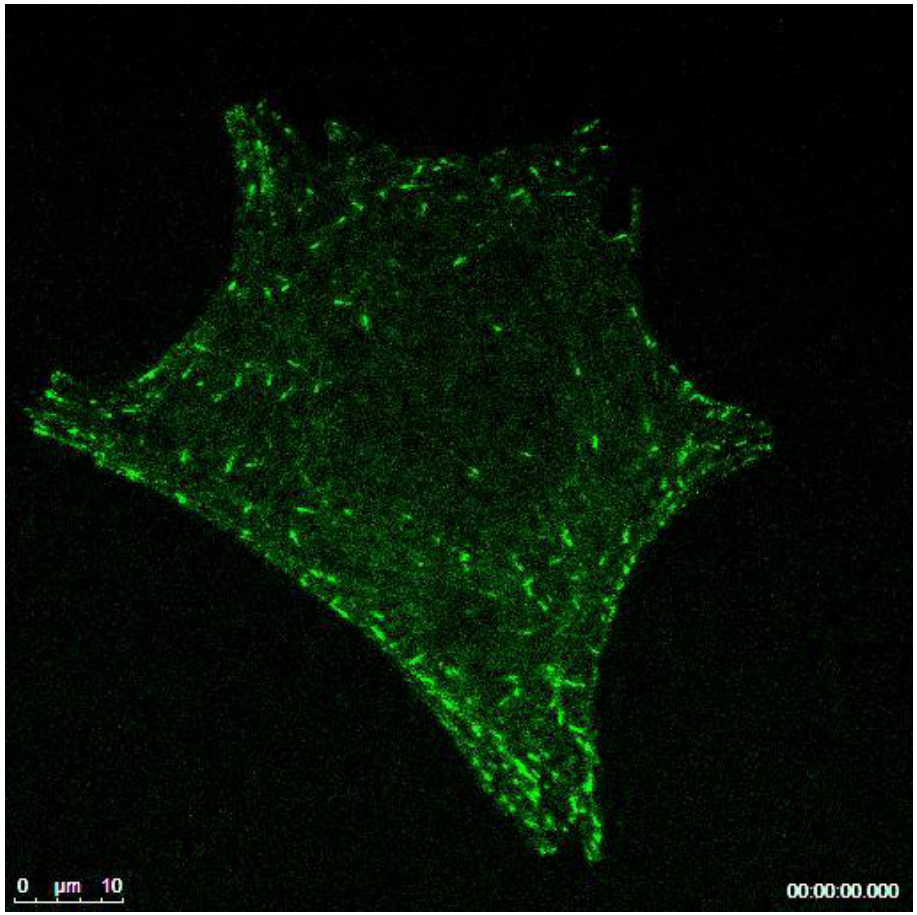


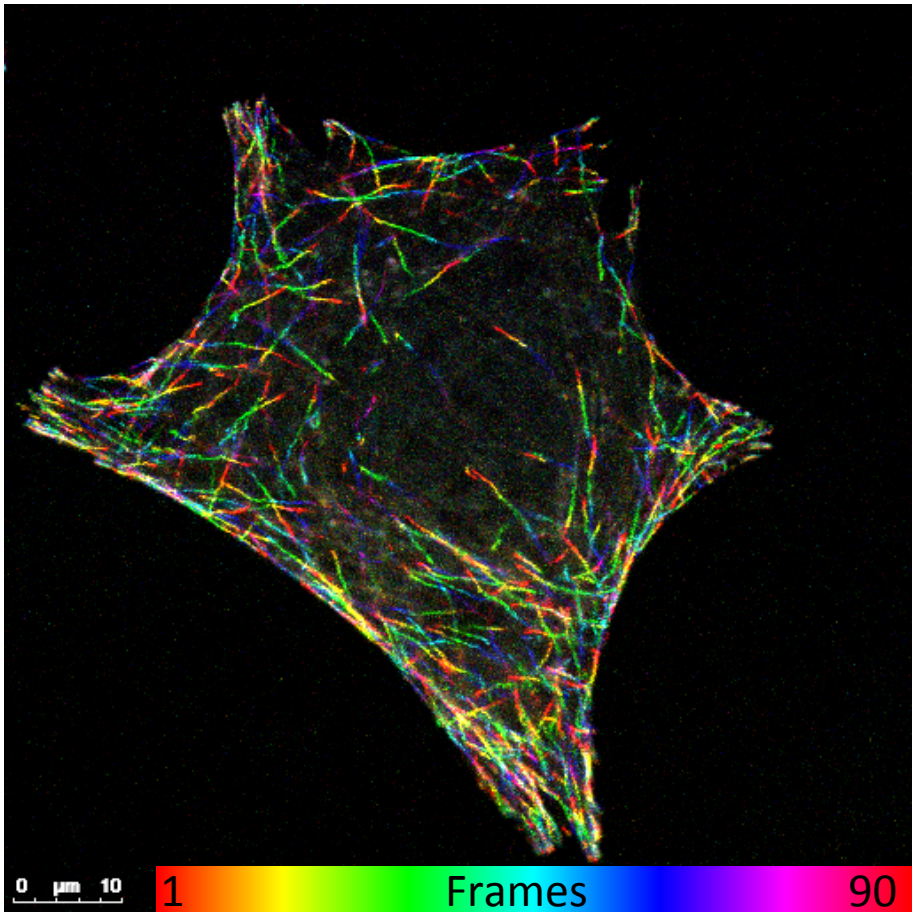


EB end tracking and dynamic instability are mechanistically linked

Detection and Tracking of EB3-GFP

Computational measurement of MT dynamics

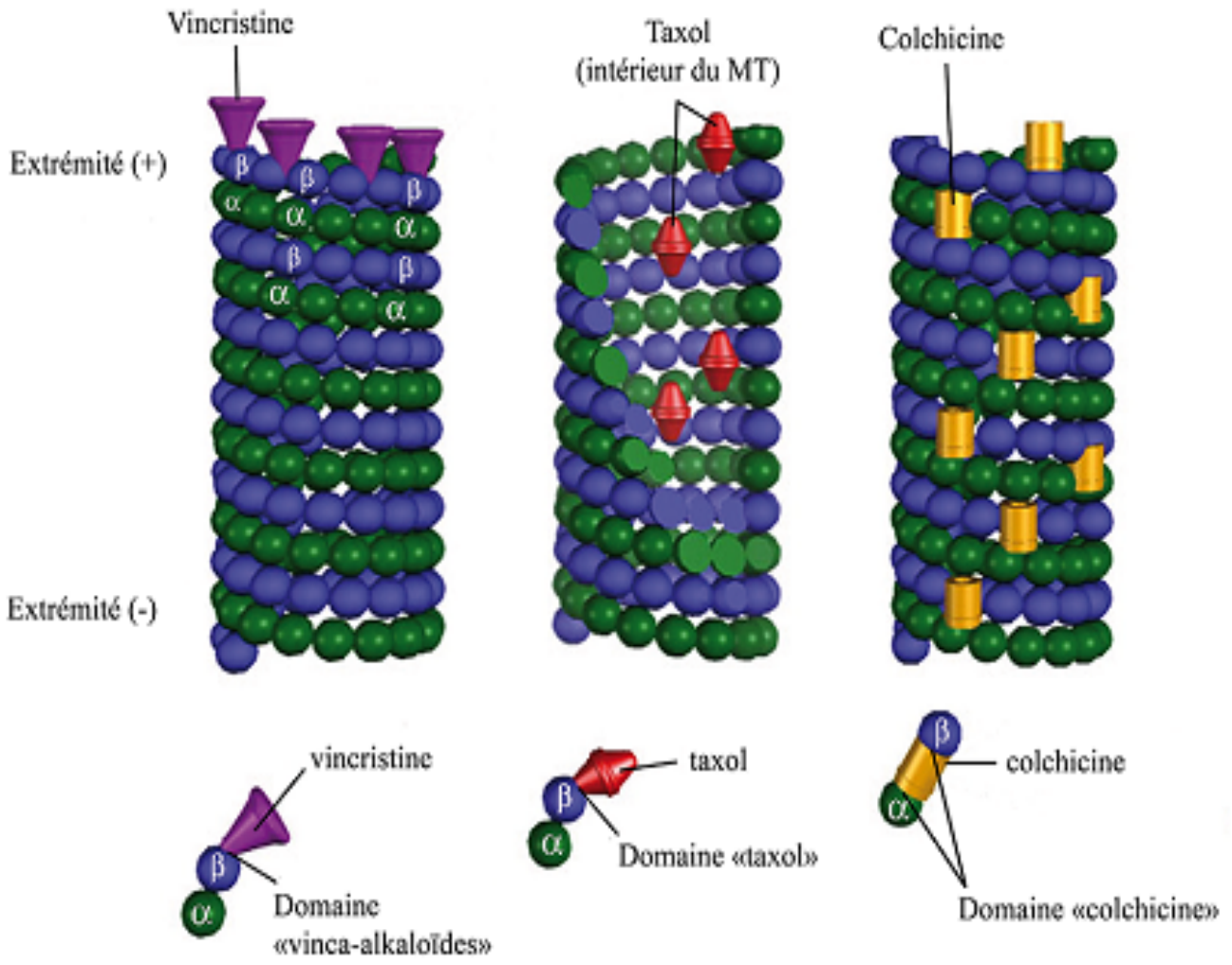




The Microtubule Tageting Agents

Classification depending on the binding site on tubulin

- **vinca-alkaloïds**
  - MT destabilisers
- **Taxanes**
  - MT stabilisers
- **Colchicine**
  - MT destabilisers



Microtubule/Tubulin as an Anticancer Target

Microtubule DESTABILIZERS

Vinca Alkaloïds
Vinblastine
Vinflunine
Vinorelbine
Vincristine

Halichondrin B analog
Eribulin

Colchicinoïds

CLASSIFIED ACCORDING THEIR EFFECTS AT **HIGH CONCENTRATIONS**

"Drug concentration references are based on the Inhibiting concentration (IC) on a 72h of cell proliferation assay"

Microtubule STABILIZERS

Taxanes
Paclitaxel
Docetaxel
Carbazitaxel

Epothilones
Patupilone

LOW and NON CYTOTOXIC CONCENTRATIONS

IC 20-30

INTERMEDIATE CYTOTOXIC CONCENTRATIONS

IC 80 or more

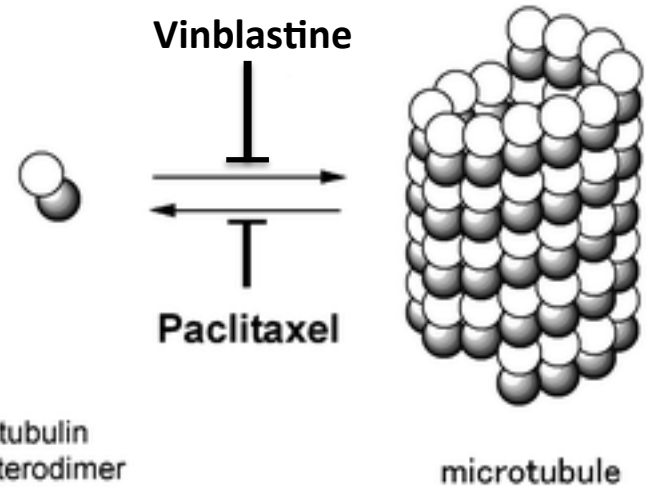
HIGH and CYTOTOXIC CONCENTRATIONS

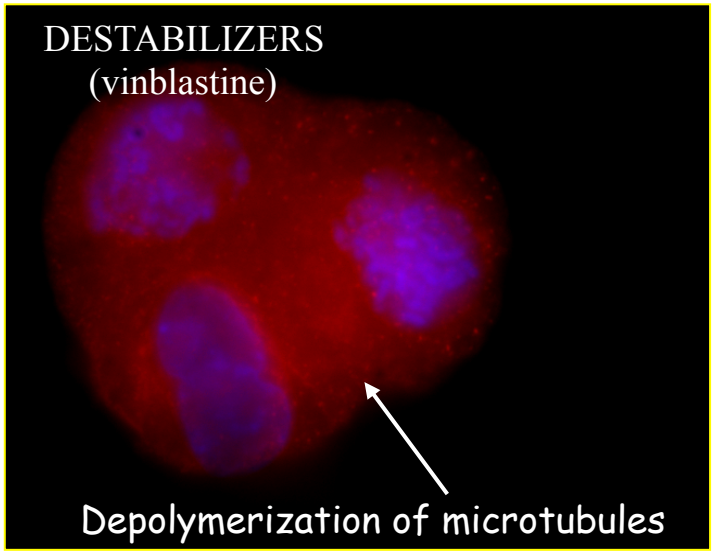
Affect interphase Functions +++ (i.e. Cell Migration etc…) Cytostatic / Reproductive death

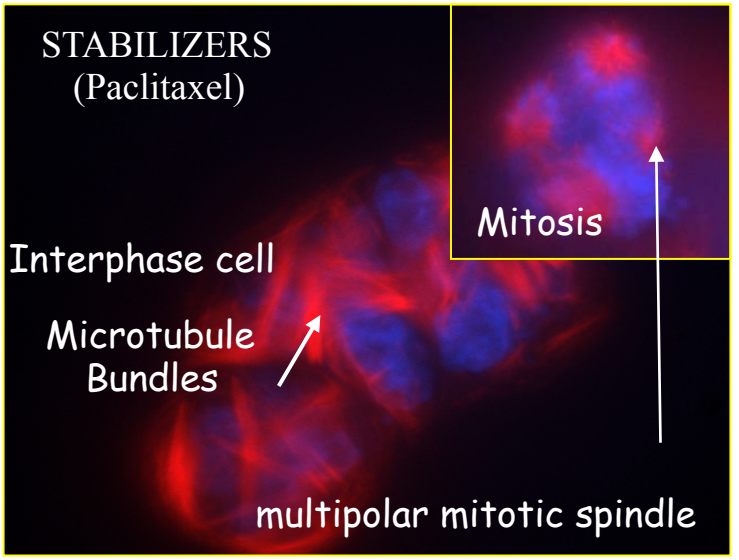
Mitotic Arrest and Apoptosis

Mitotic Arrest and Apoptosis

At “high” concentrations microtubule-targeting agents (MTA) affect the microtubule polymer mass







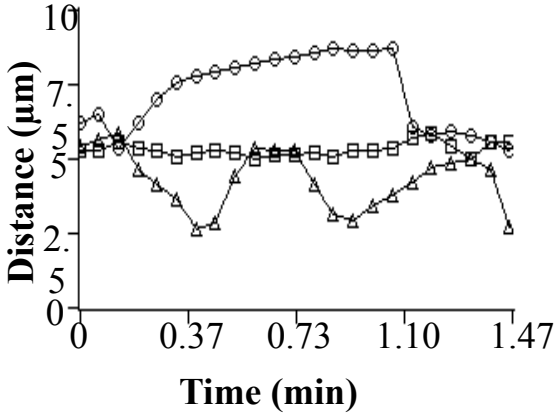
Personnal data

MTA suppress MT dynamic instability at intermediate concentrations and increase it at low concentrations

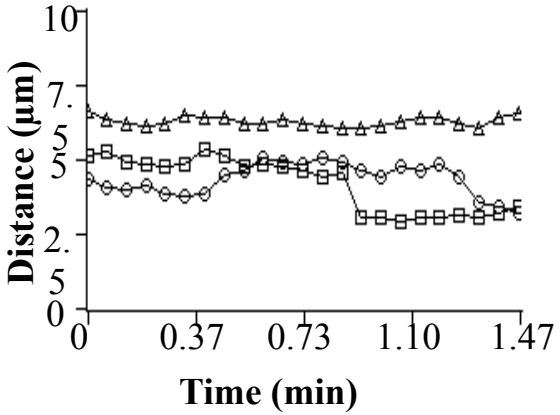
INTERMEDIATE

LOW

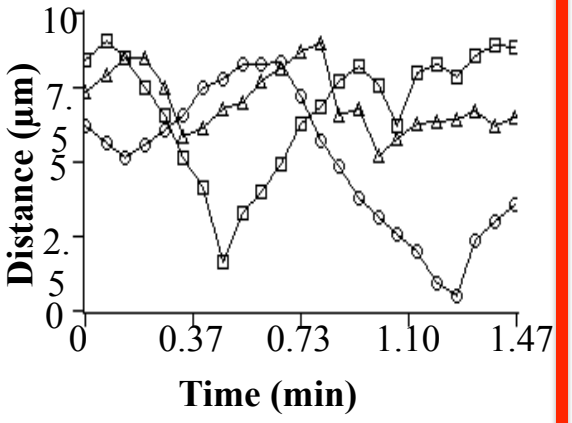
Control



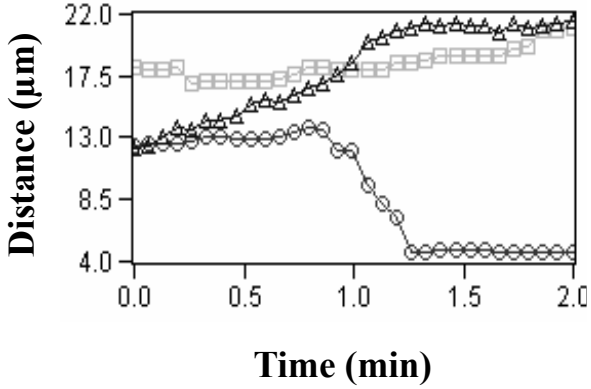
Paclitaxel 100 nM



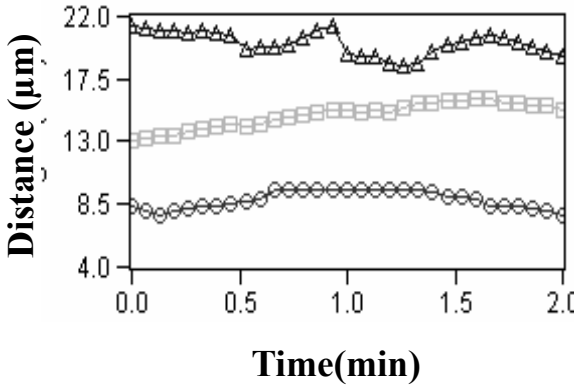
Paclitaxel 5 nM



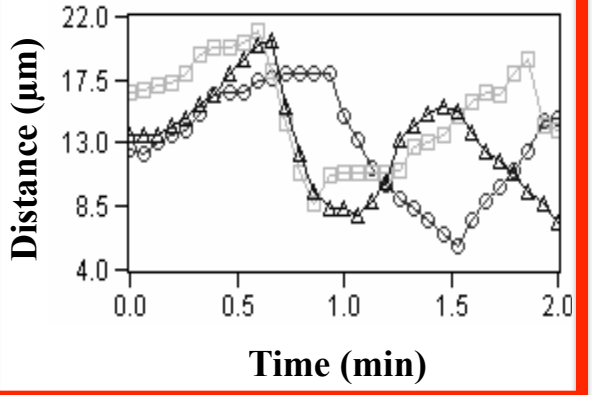
Control



Vinflunine 20 nM



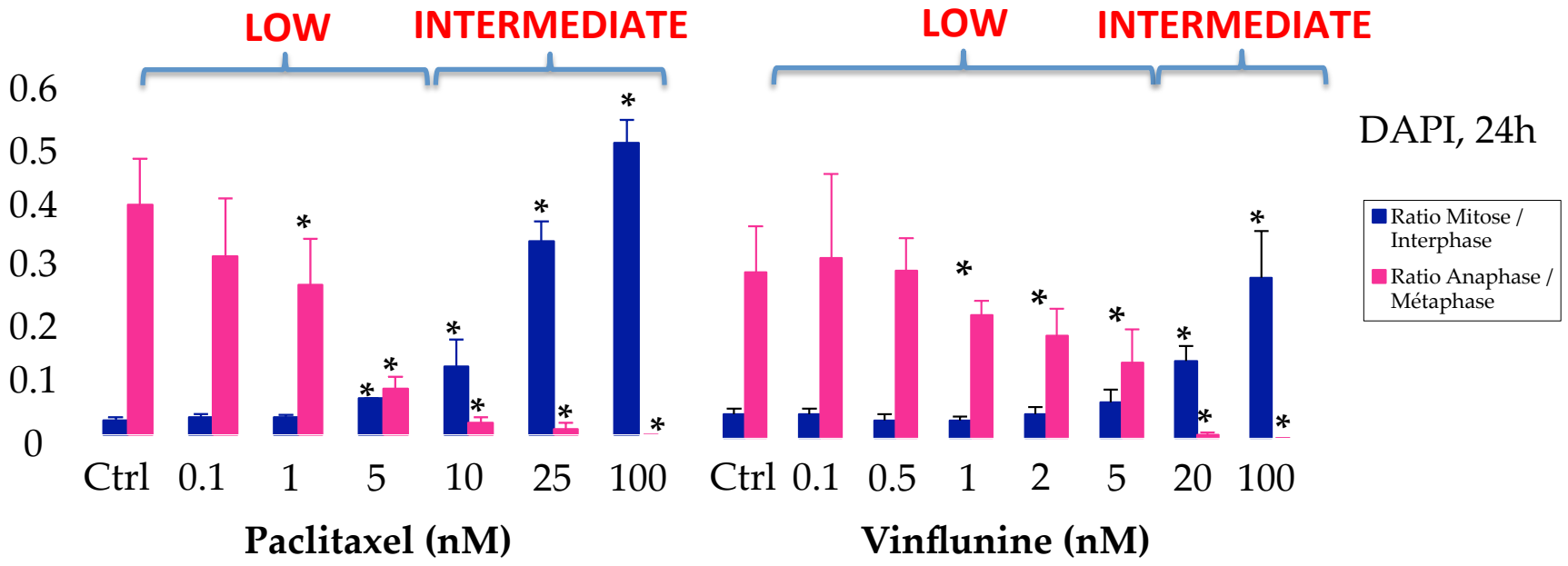
Vinflunine 2 nM

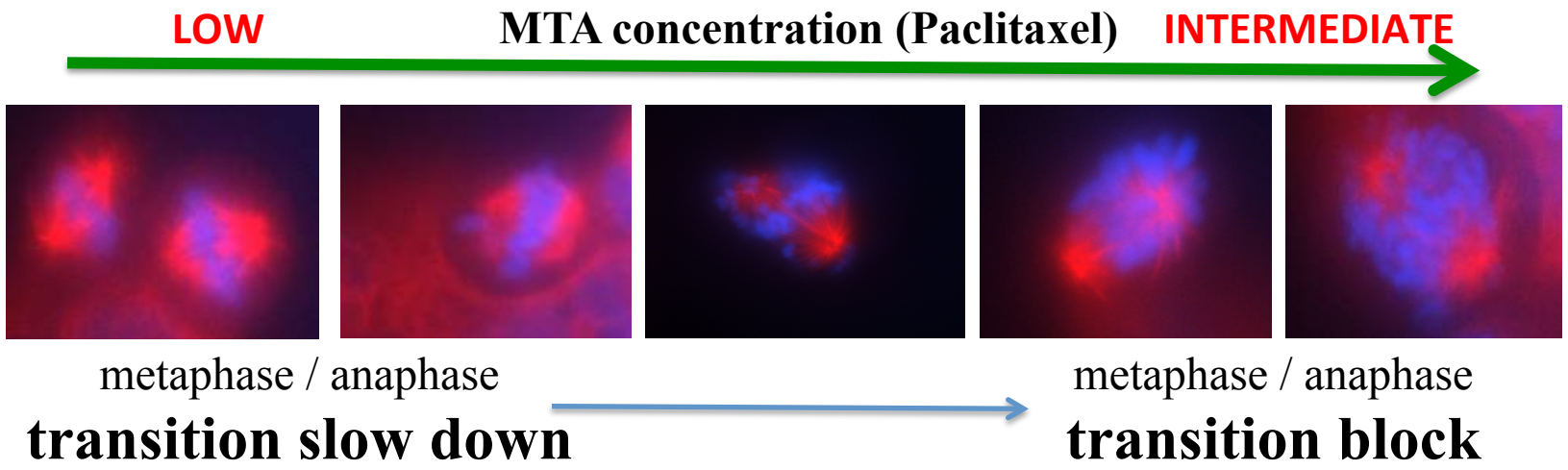


Pasquier et al, *Cancer Res* 2005

Pourroy et al., *Cancer Res* 2006

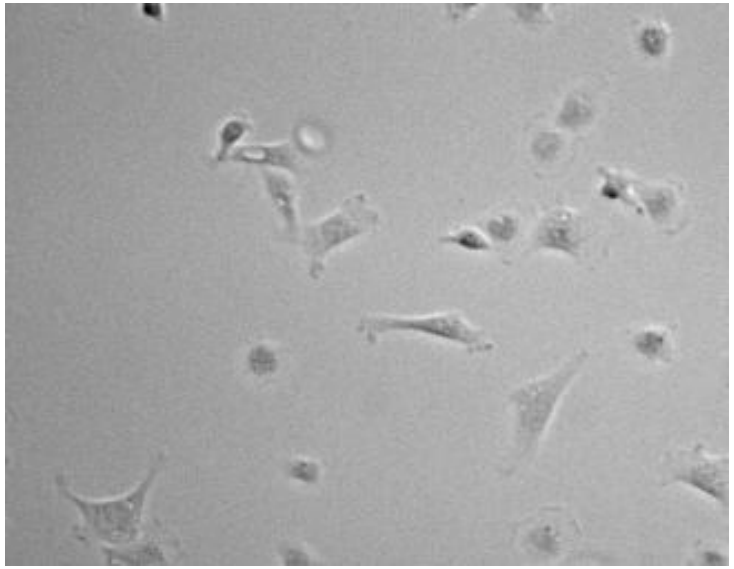
Low and intermediate concentrations of MTA differently affect mitotic progression



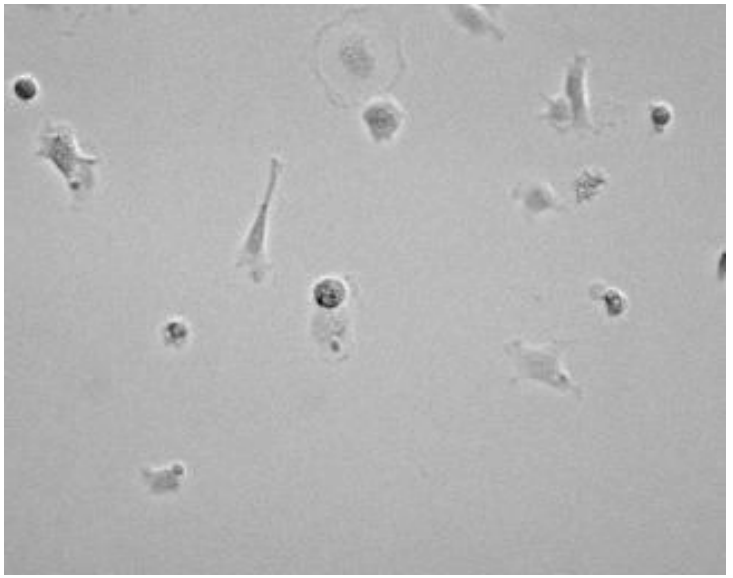


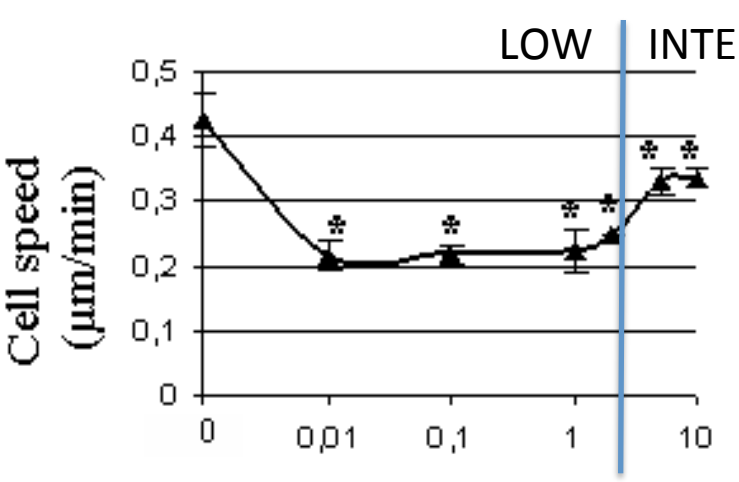
Low concentrations of MTAs inhibit cell motility

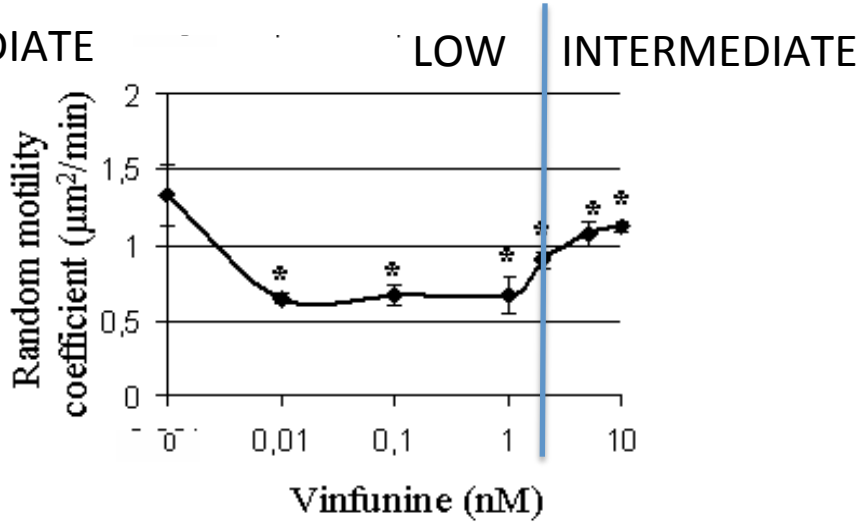
Control



Vinflunine 1 nM





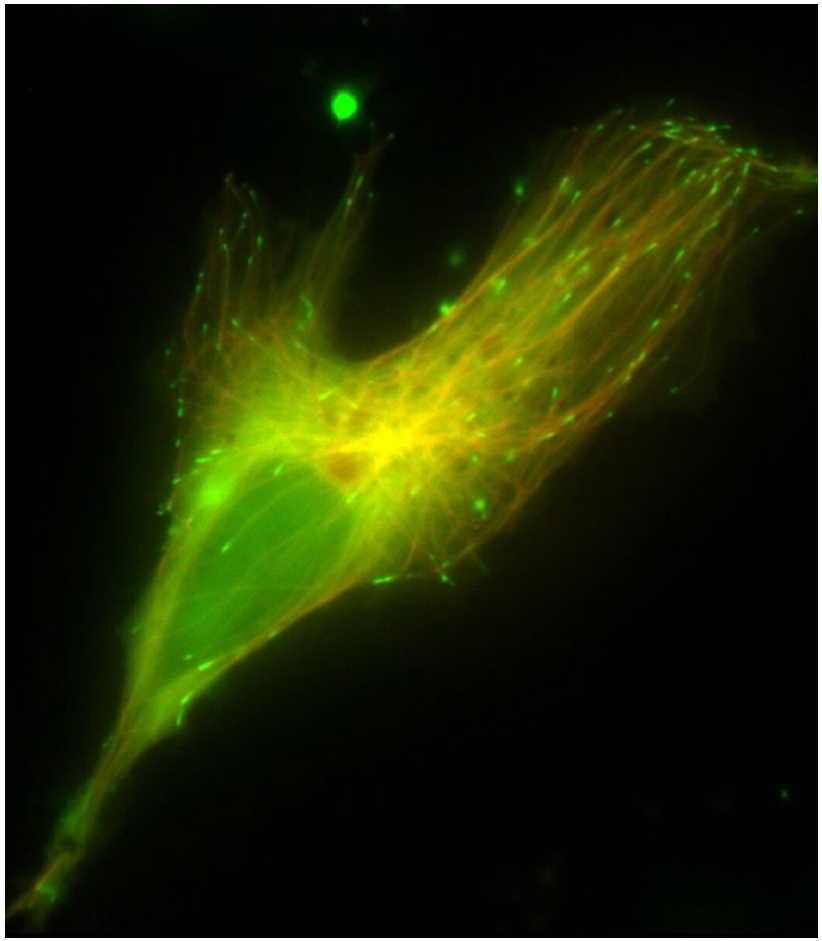


Honoré et al., 2008

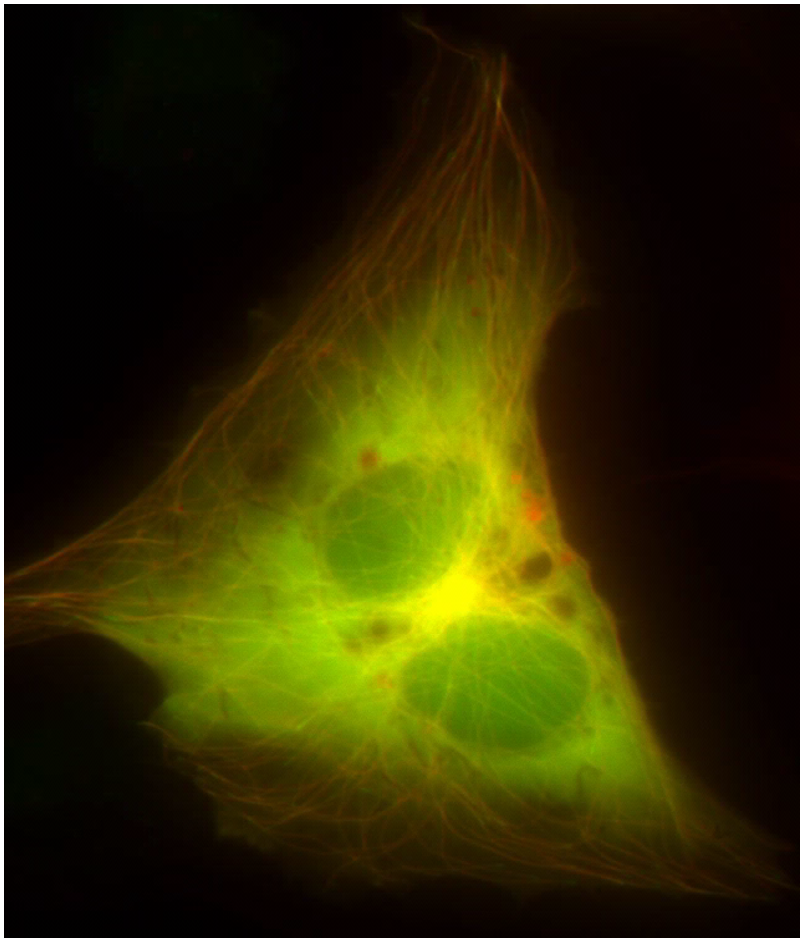
vinflunine inhibits EB1 accumulation at microtubule + End

Red: Tubulin-mcherry
Green: EB1-GFP

CONTROL



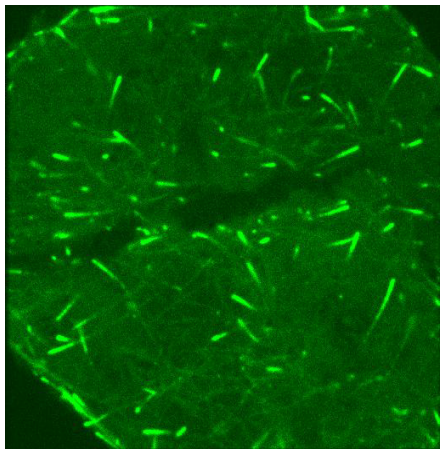
Vinflunine 1 nM



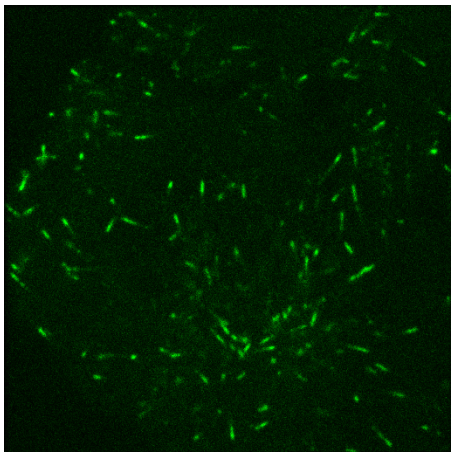
INCREASED MT DYNAMICS
NO VISIBLE EB1 COMETS

Honoré et al., 2008

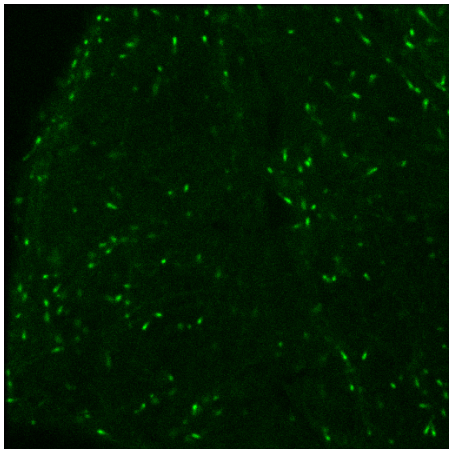
Patupilone inhibits EB1 accumulation at microtubule + Ends



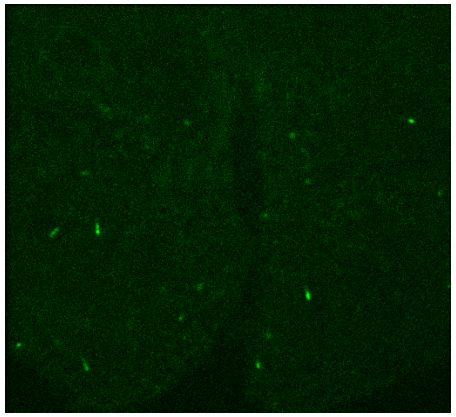
CONTROL



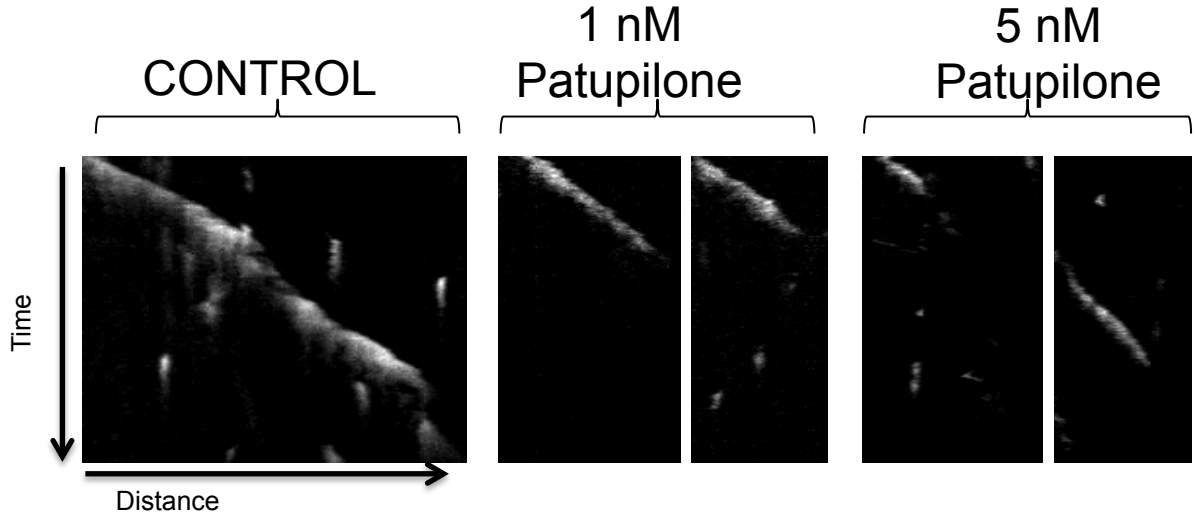
1 nM Patupilone



5 nM Patupilone

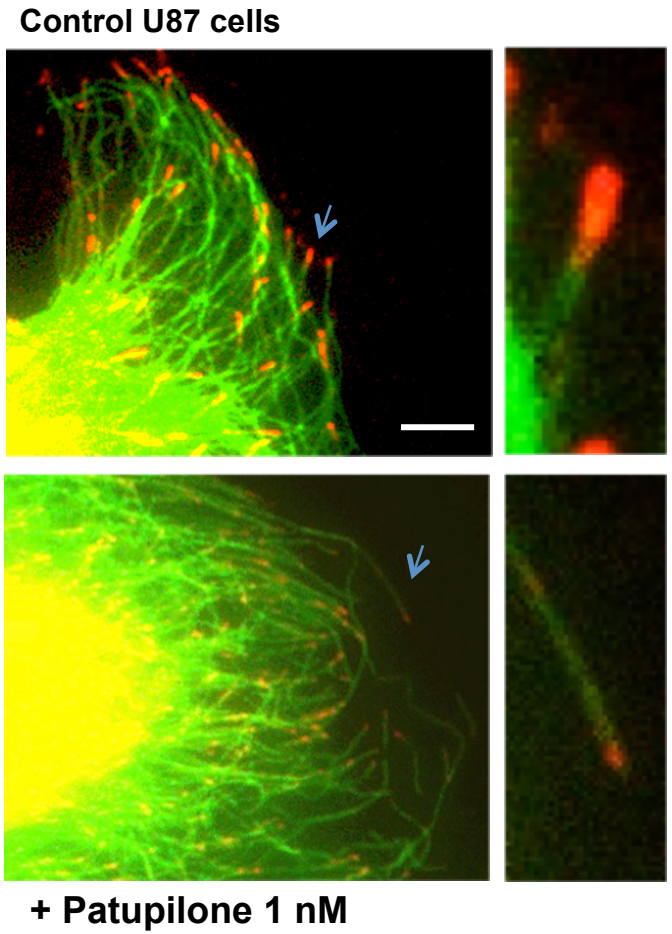


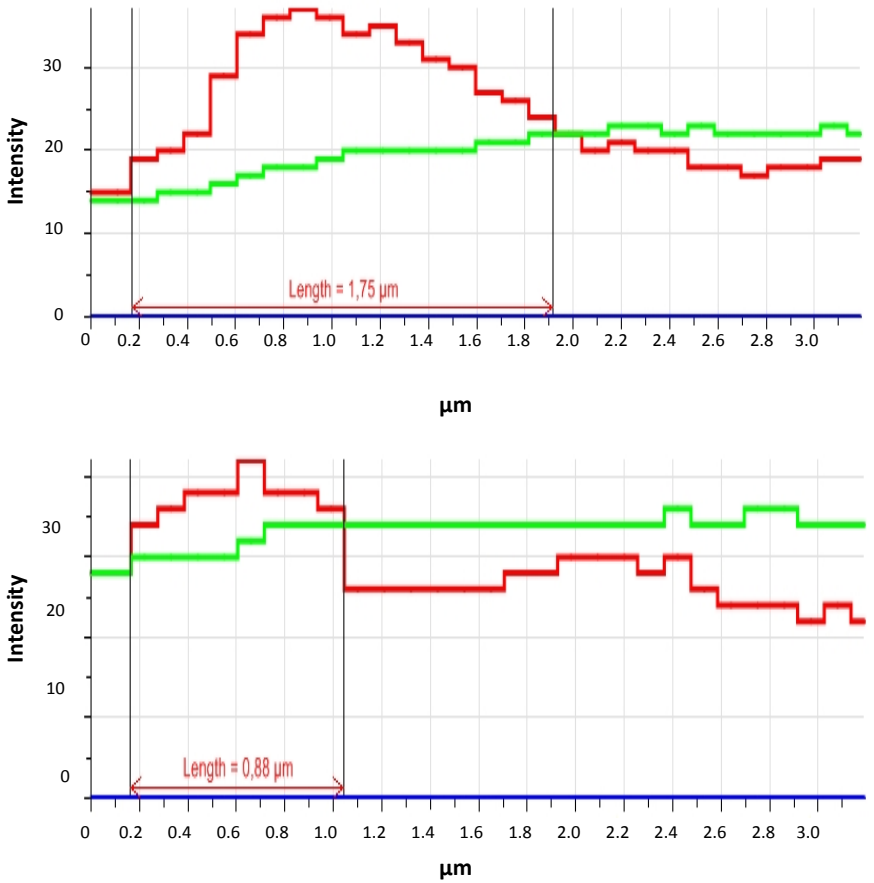
10 nM Patupilone



Low concentrations of patupilone inhibit EB1 accumulation at MT + end in cells

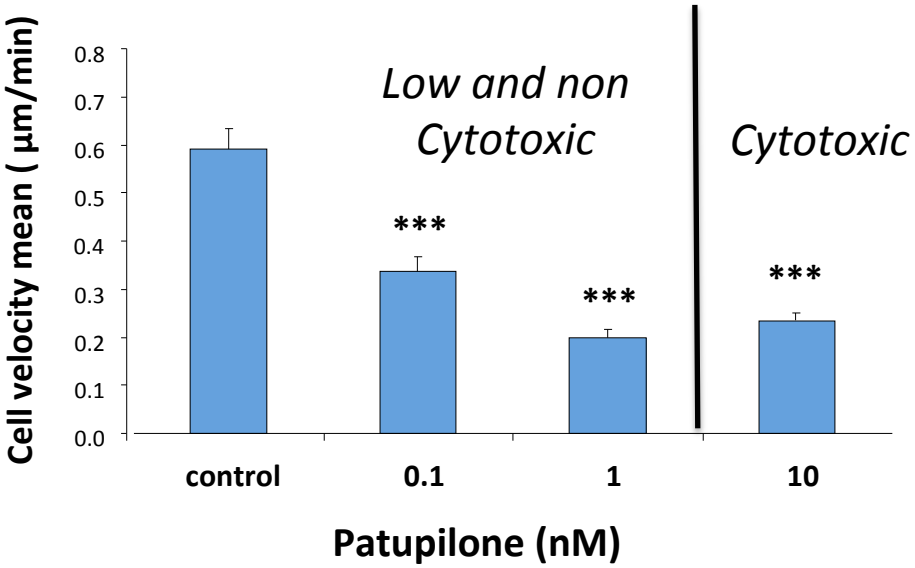
Pagano et al., *Biochem Pharmacol* 2012

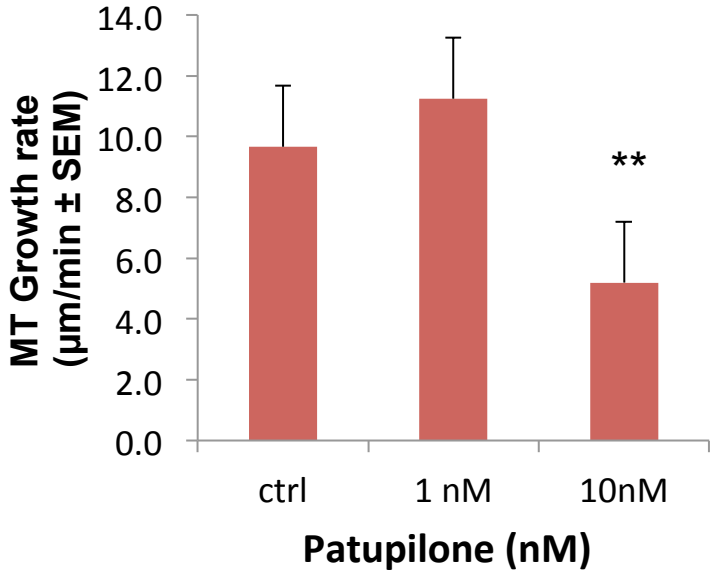


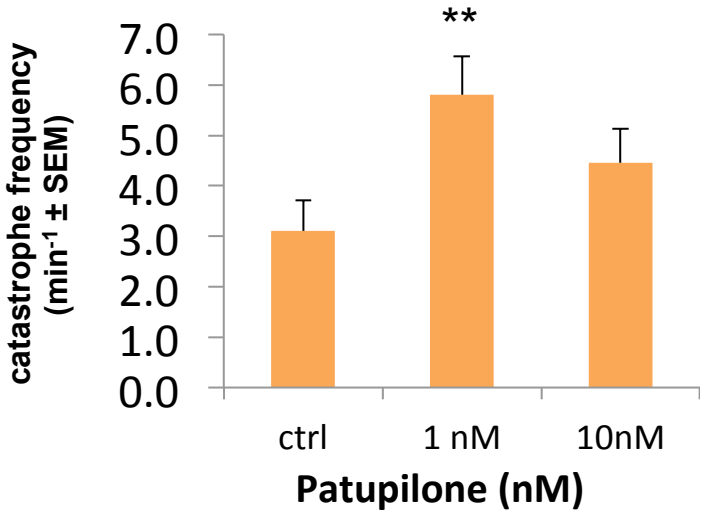


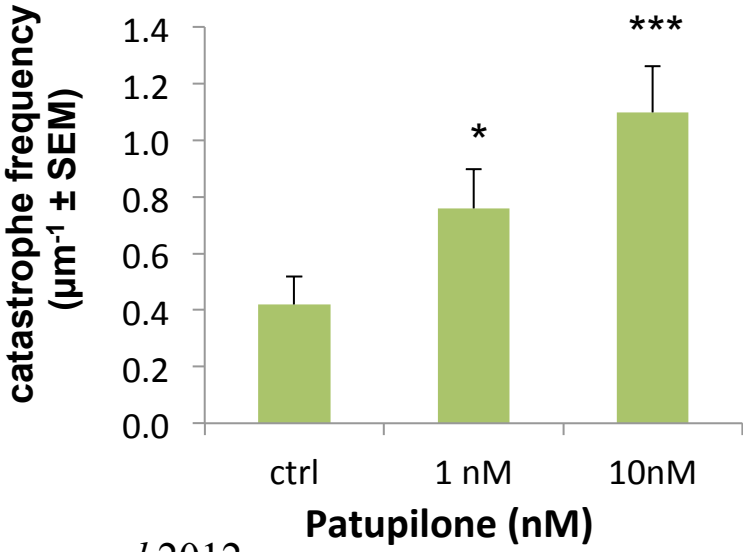
Does the decrease of the EB stabilizing cap favor MT catastrophes in cells?

Patupilone anti-migratory effect is linked to the induction of MT catastrophes in U87 Glioblastoma cells





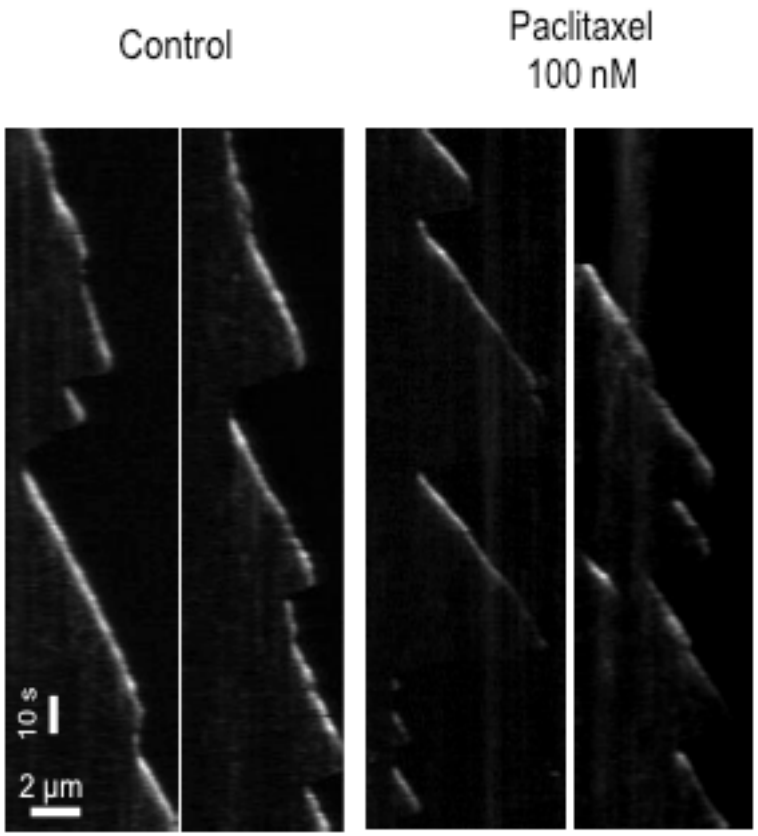




Pagano et al., *Biochem Pharmacol* 2012

Low nanomolar concentration **paclitaxel increased MT dynamics *in vitro in presence of EBs***

Pagano et al., *Biochem Pharmacol* 2012



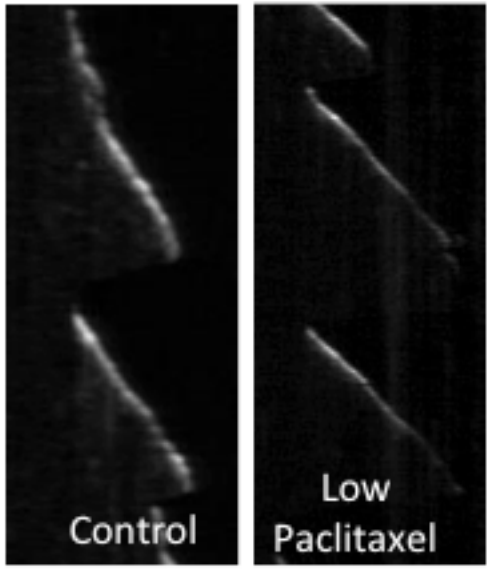
	GR (um/min)	SR (um/min)	Cata fr (per min)	Rescue fr. (per min)	n
Control	3.87 ± 1.0	19.09 ± 16.0	1.72 ± 0.1	2.12 ± 0.3	62
Paclitaxel 1 nM	4.13 ± 1.4	17.87 ± 11.2	2.10 ± 0.1	3.79 ± 0.4	37
Paclitaxel 10 nM	4.89 ± 1.5	21.30 ± 15.8	2.62 ± 0.2	5.77 ± 0.6	38
Paclitaxel 100 nM	5.99 ± 1.5	28.12 ± 25.8	2.96 ± 0.1	4.57 ± 0.5	50

In vitro tip tracking assay
EB3-GFP-Kymographs

EBs sensitize MT to the action of MTAs and allow such peculiar increased dynamics at low concentrations

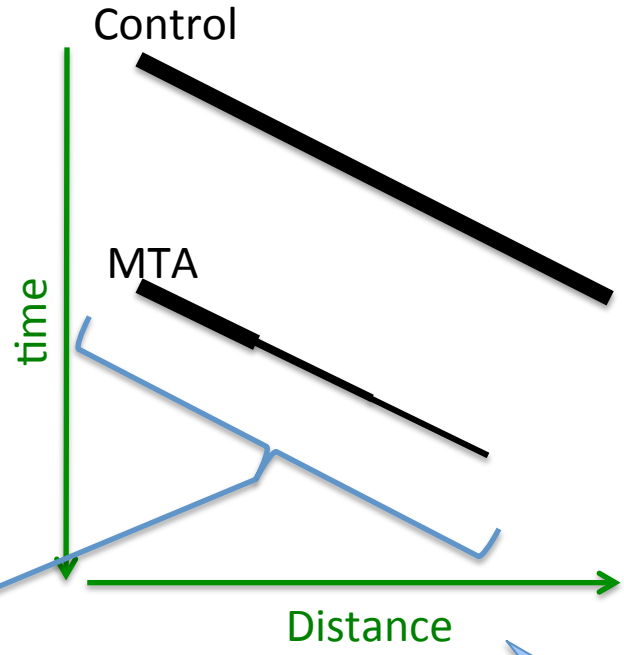
MTAs affect MT aging

Kymographs
of EB3-GFP tip tracking assay



-28%

Mean FI
(% change)

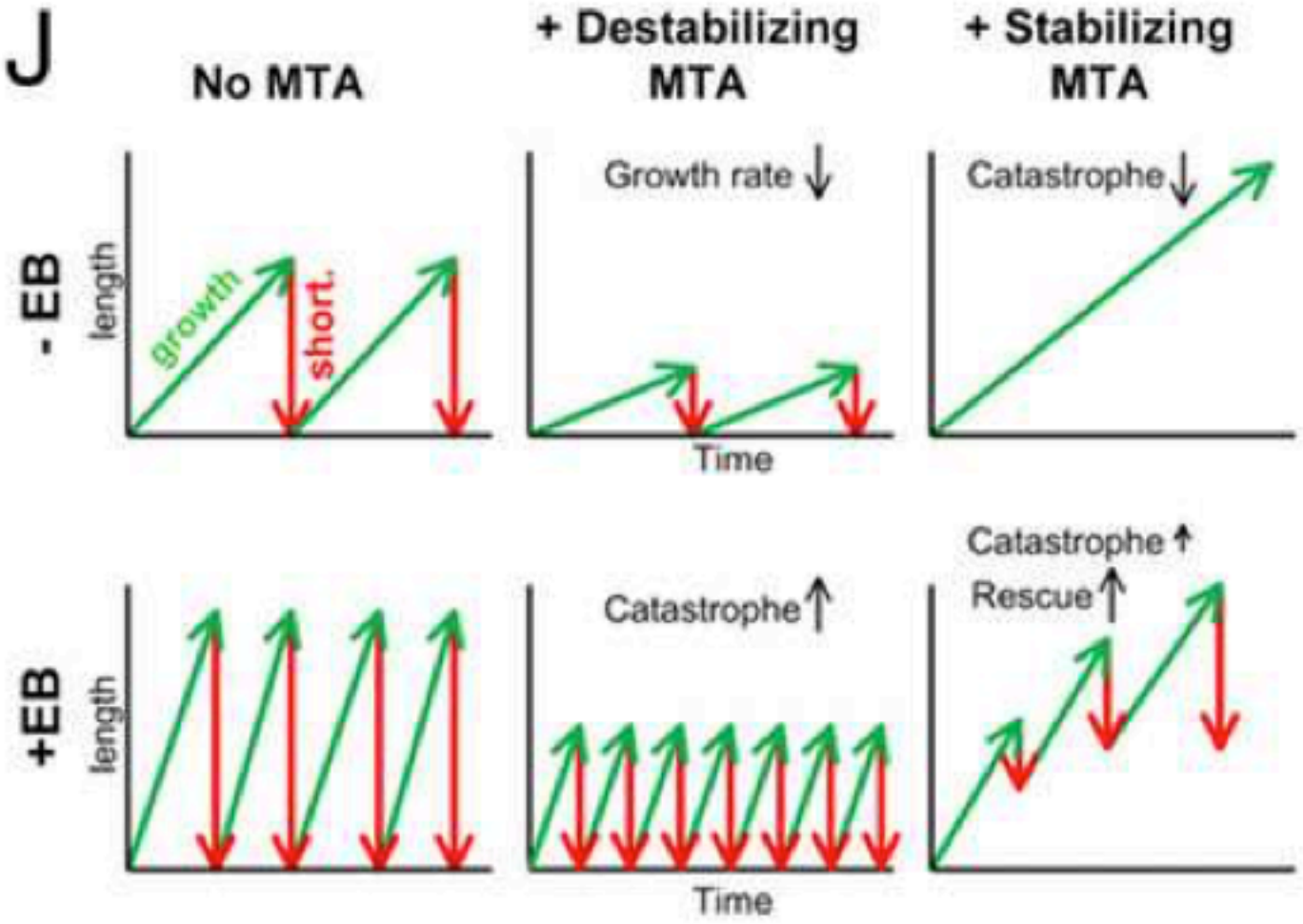


Microtubule « aging »
Time dependent decrease of the stabilizing cap
(Hypothesis = time dependent GTP hydrolysis)

Deterministic mathematical model

Poster
Ayuna
Barlukova

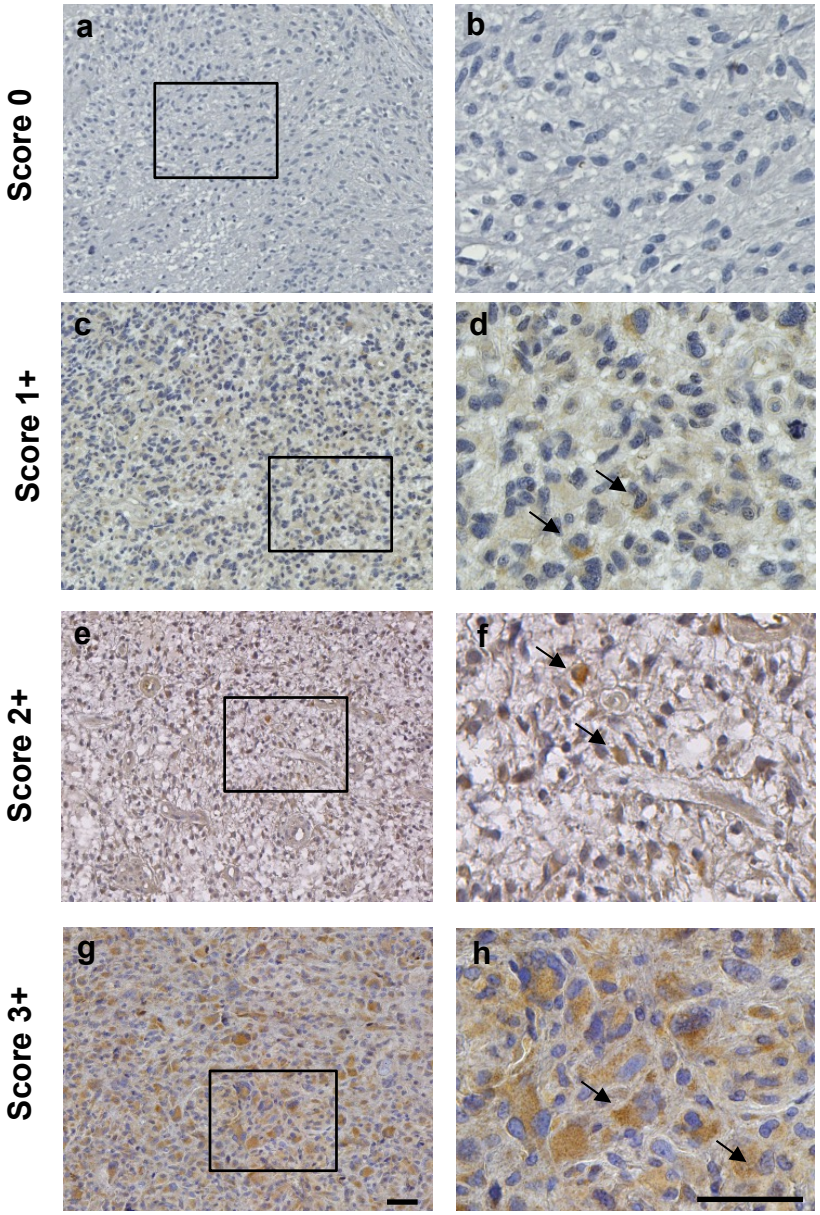
Effect of drugs differ according the presence or the absence of EB proteins

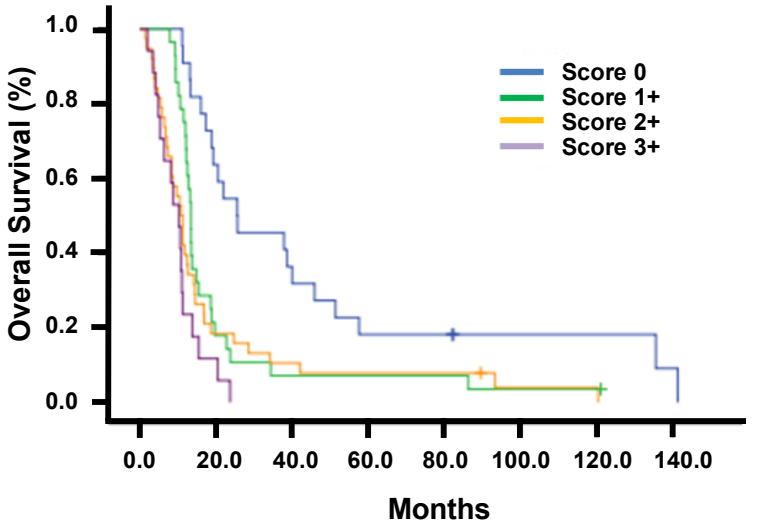


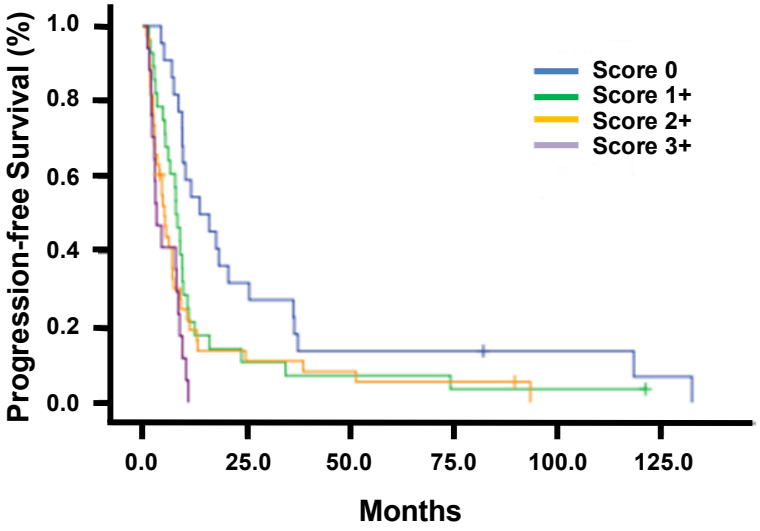
Mohan et al., 2013

EB1 a bad prognostic marker in Glioblastoma

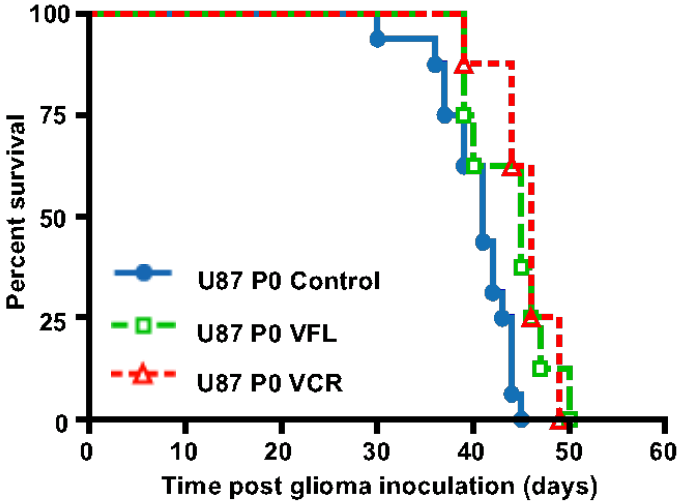
EB1 staining in human GBM patient (n=109)



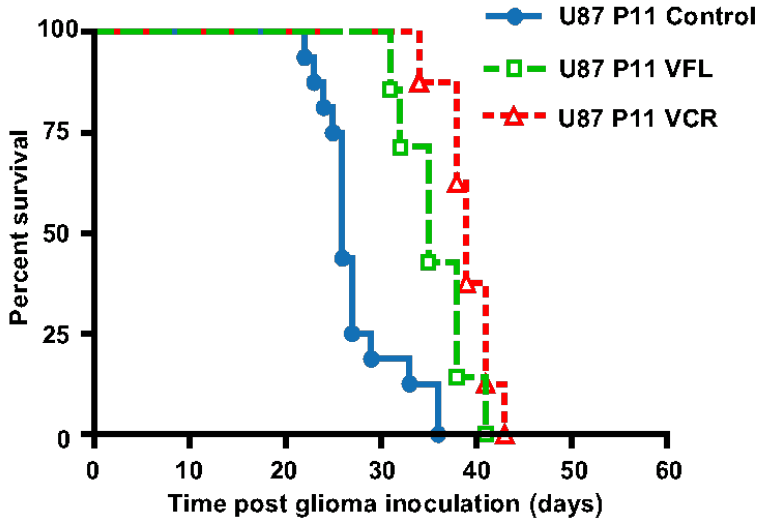




EB1 overexpression sensitize to MTAs *in vivo*



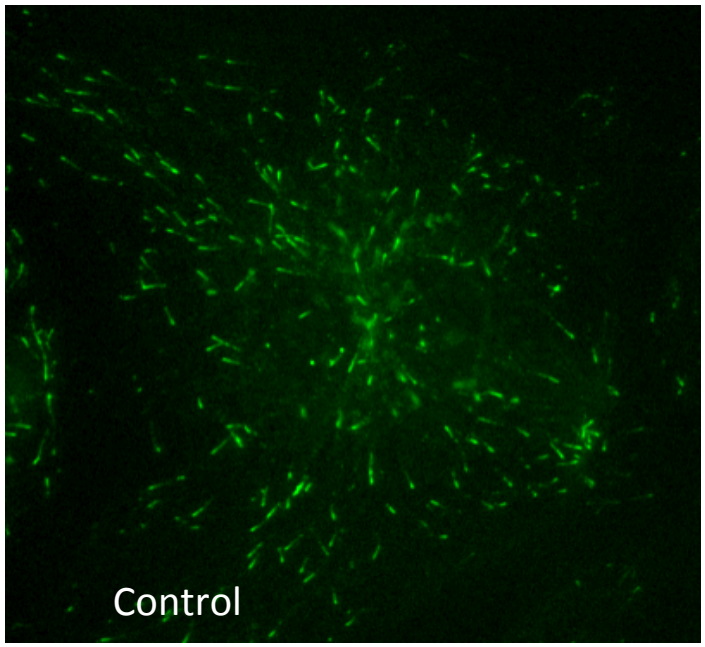
Low level of EB1 in Glioblastoma

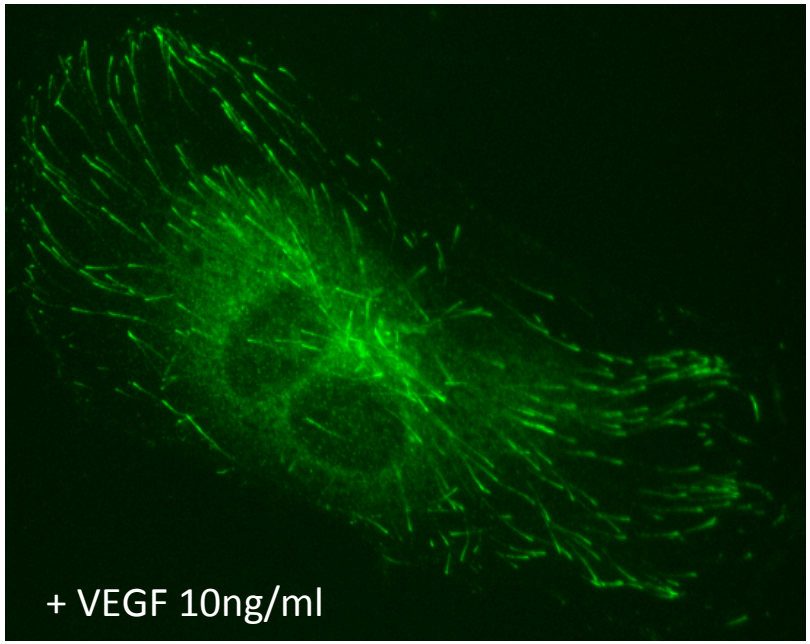


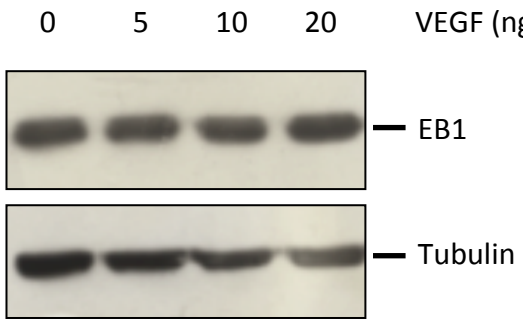
High level of EB1 in Glioblastoma

EB1 DETYROSINATION

Pro-migratory VEGF increased EB1 comet length in endothelial cells

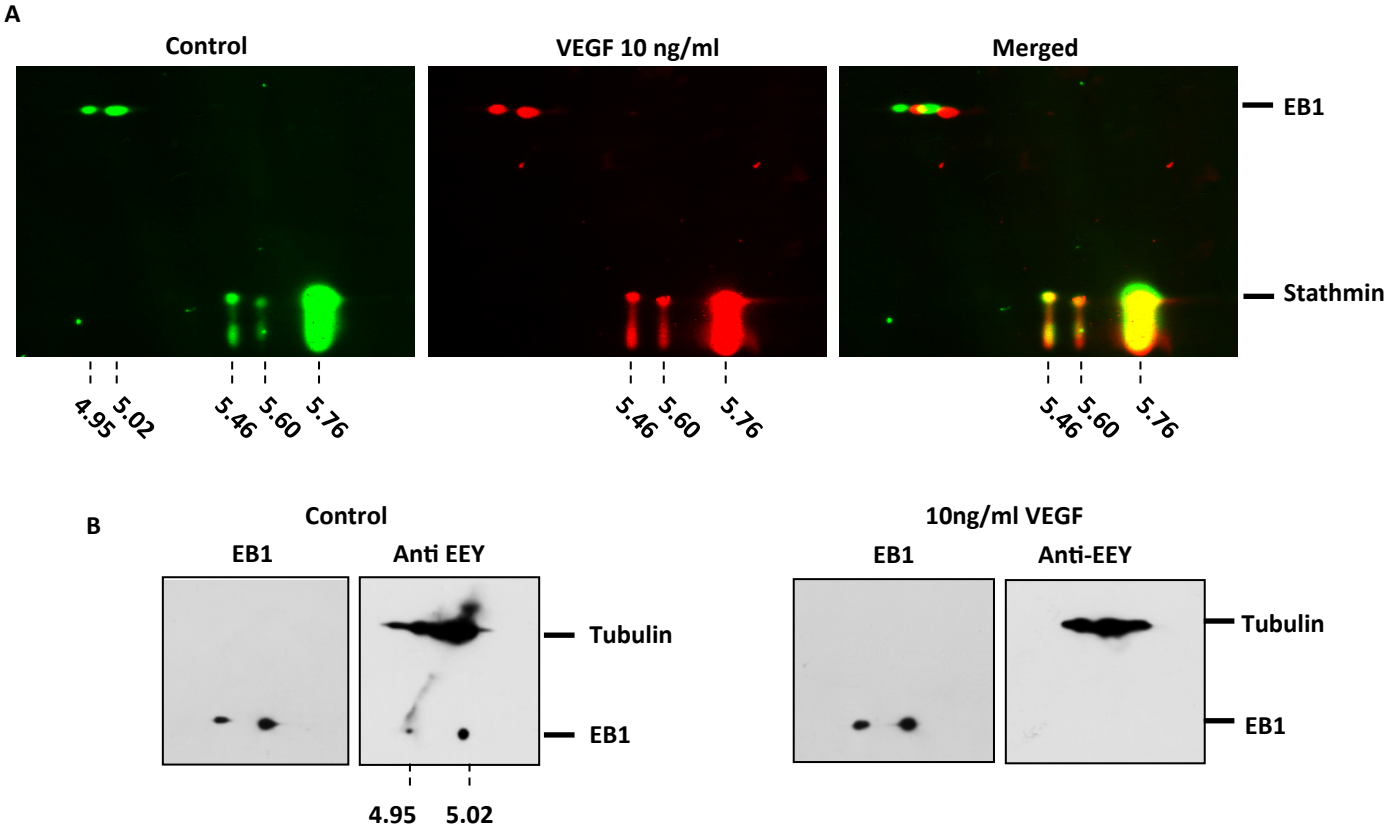






Rovini et al., Plosone2013

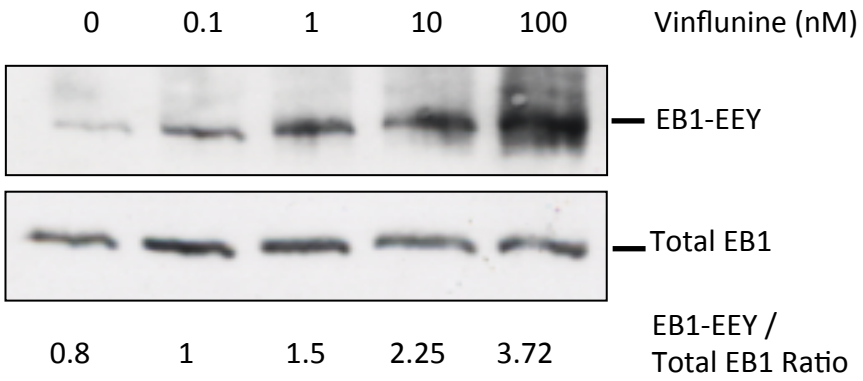
Pro migratory VEGF induced EB1 detyrosination

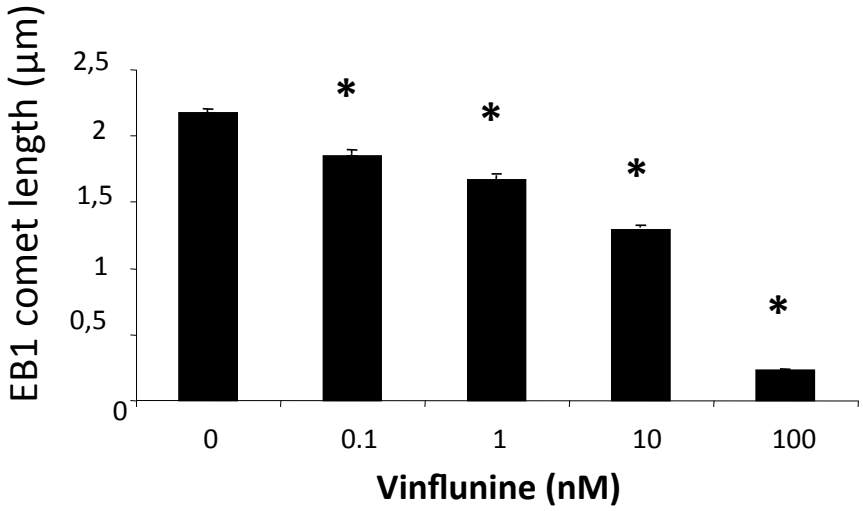


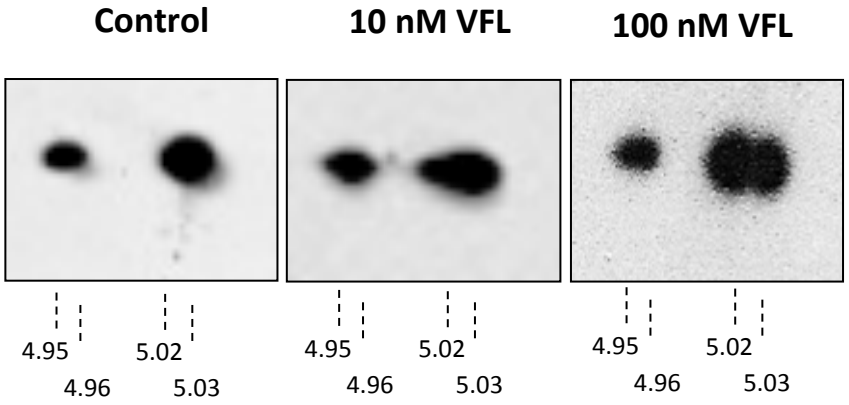
VEGF

Rovini et al., Plosone2013

Vinflunine abolishes VEGF effect: it induces EB1 retyrosination and decreases EB1 comet length





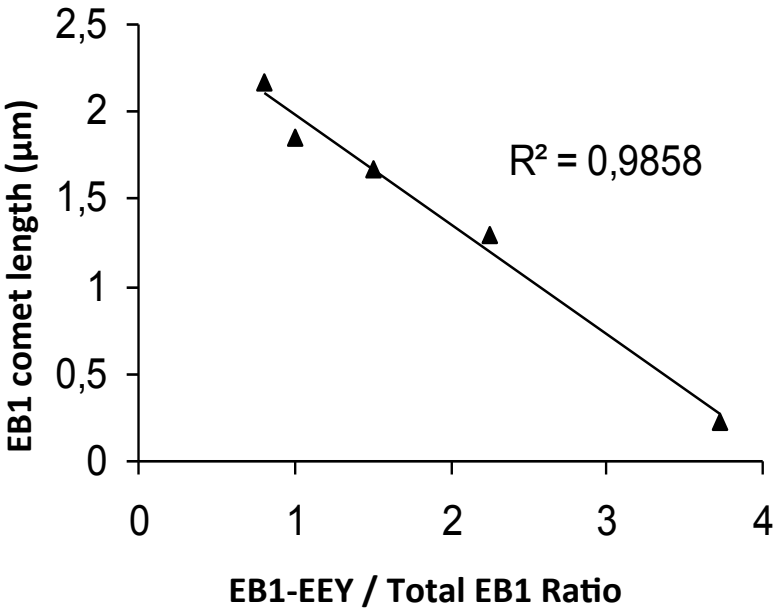


VEGF

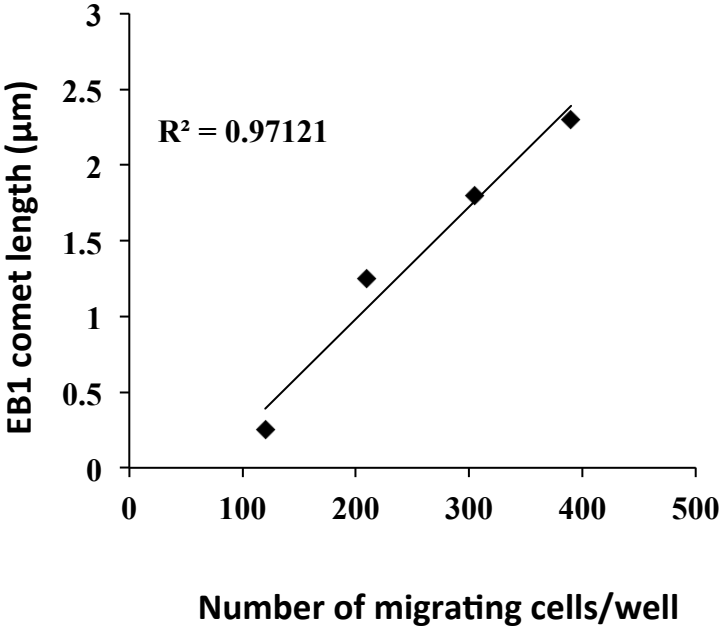
VINFLUNINE

Anti-migratory effect of Vinflunine is correlated with inhibition of EB1 accumulation at MT + end and EB1(re)tyrosination

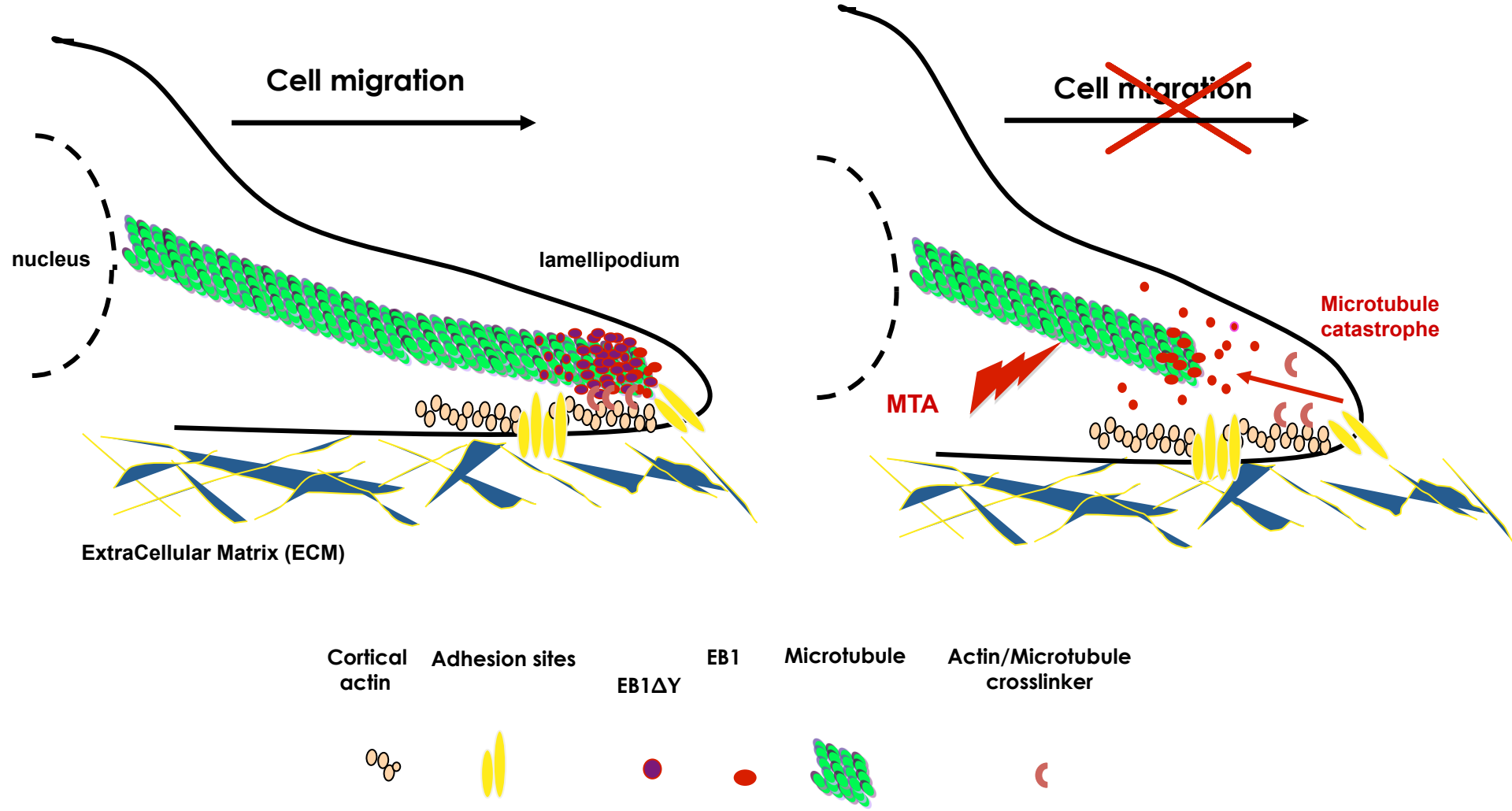
Correlation between EB1 comet length and EB1-EEY

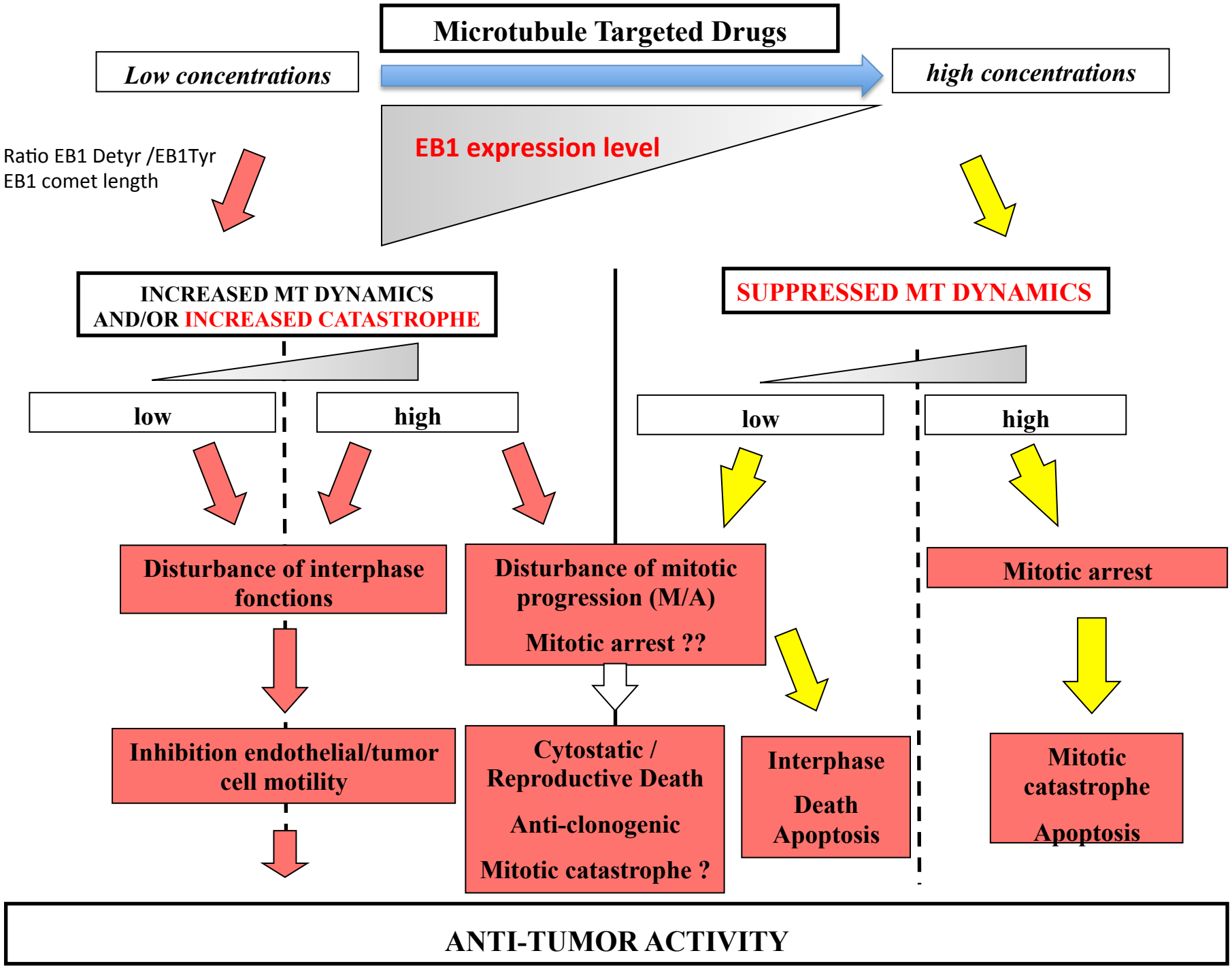


Correlation between EB1 comet length and cell migration



Model for anti-migratory effect of MTAs




Microtubule Targeted Drugs
Low concentrations
high concentrations
EB1 expression level
Ratio EB1 Detyr /EB1Tyr
EB1 comet length
INCREASED MT DYNAMICS
AND/OR INCREASED CATASTROPHE
SUPPRESSED MT DYNAMICS
low
high
low
high
Disturbance of interphase fonctions
Disturbance of mitotic progression (M/A)
Mitotic arrest ??
Mitotic arrest
Inhibition endothelial/tumor cell motility
Cytostatic / Reproductive Death
Anti-clonogenic
Mitotic catastrophe ?
Interphase
Death
Apoptosis
Mitotic catastrophe
Apoptosis
ANTI-TUMOR ACTIVITY

Centre de
Recherche en
Oncologie biologique et
Oncopharmacologie

Thank you for your attention!

INSERM U911 – Team 1- MARSEILLE

EQUIPE 1

D.BRAGUER
V.BOURGAREL
M.CARRE
E.PASQUIER
M-A.ESTEVE
M-P.MONTERO
N.ANDRE
M.LEGRAND
F. CORREAD
S.HONORE
R.BERGES
M.PETIT
S.ODDOUX
C.RICARD

EQUIPE 4

D.FIGARELLA
L.OUAFIK
N.BAEZA
O.CHINOT
E.TABOURET

I2M

F. HUBERT
C. GOMEZ
A. BENABDALLAH
R. TESSON
A.BARLUKOVA
D.WHITE