Microtubule dynamics

physiology and therapeutic targeting

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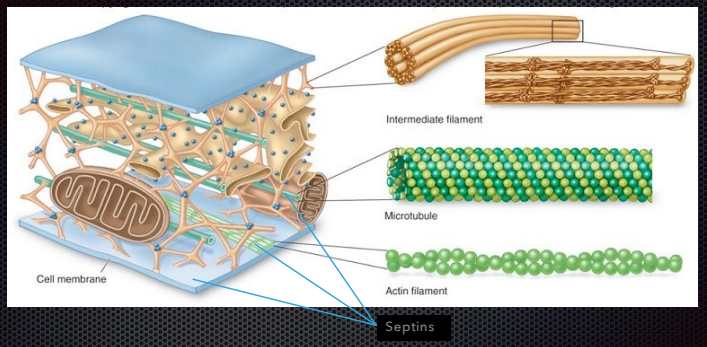
1

Components:



http://www.yellowtang.org/images/three_protein_jf_be_c_ia_784.jpg

3 main types of structures in mutual interaction and in interaction with several membrane organelles, proteins and complexes of the cytoplasm, RNA, DNA, etc...

2

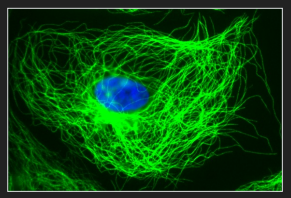
Microtubules

A network that allows the cell to :

- **explore its cytoplasm**
- **localise its constituents**

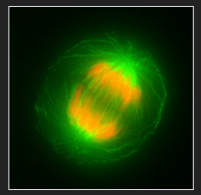
- Organelle positioning and membrane traffic
- Organisation of signalling pathways
- Polarity and migration

Interphase

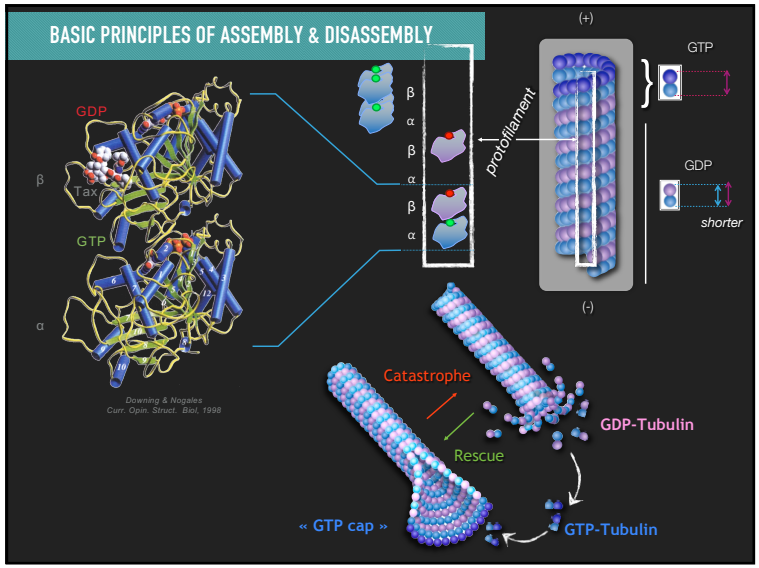


- Alignment and segregation of chromosomes

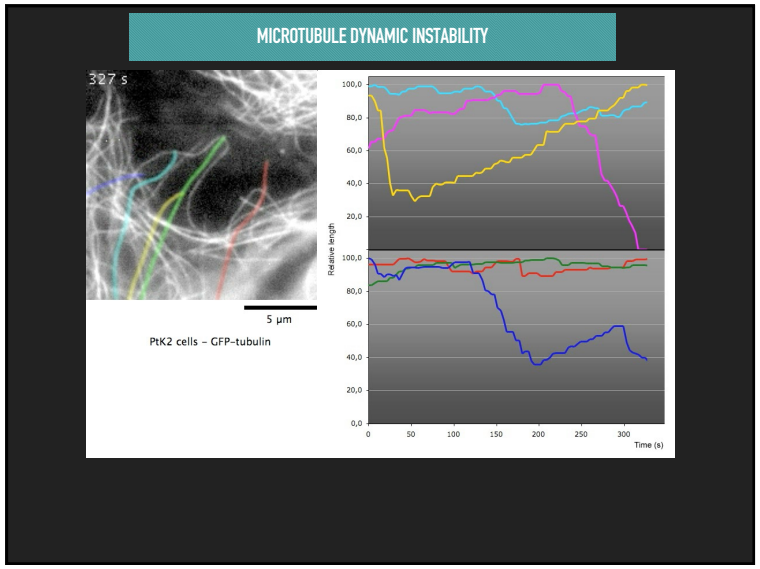
Mitosis



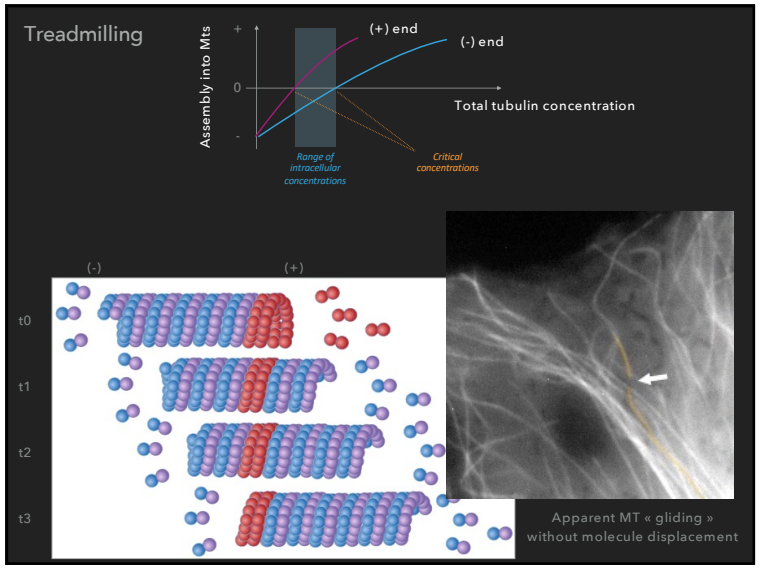
3



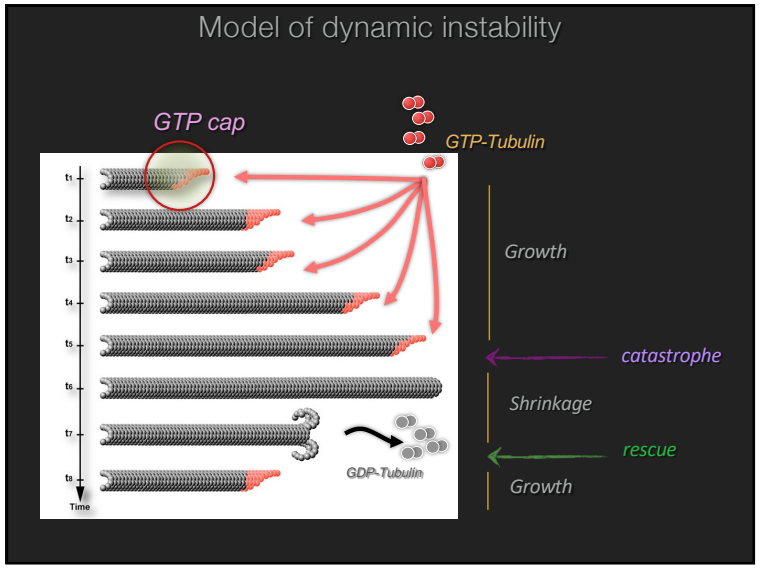
4


MICROTUBULE DYNAMIC INSTABILITY
327 s
5 µm
PtK2 cells – GFP-tubulin
Relative length
Time (s)

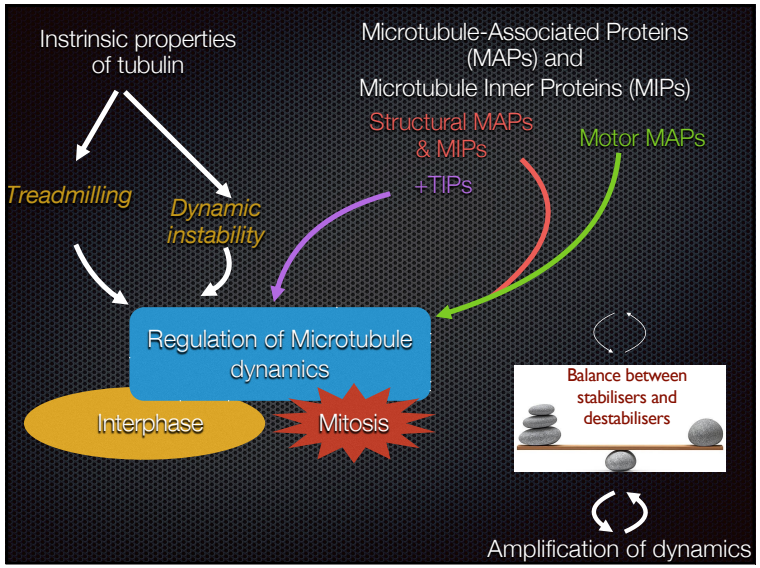
5


Treadmilling
Assembly into Mts
(+) end
(-) end
Total tubulin concentration
Range of intracellular concentrations
Critical concentrations
(-)
(+)
t0
t1
t2
t3
Apparent MT « gliding » without molecule displacement

6

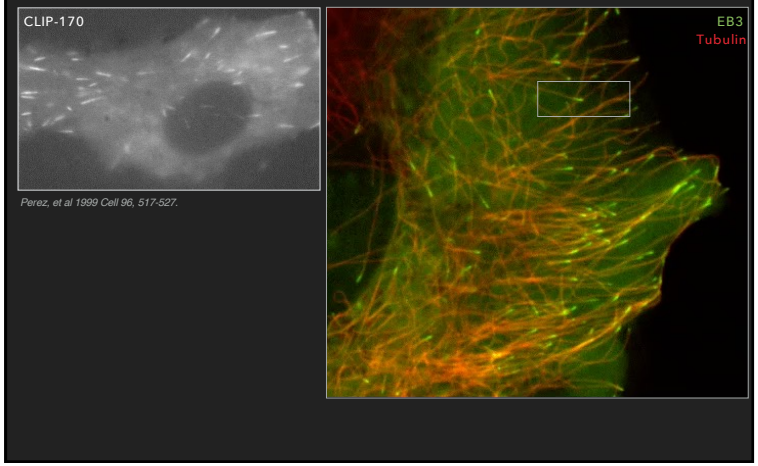

Model of dynamic instability
GTP cap
GTP-Tubulin
GDP-Tubulin
Time
Growth
catastrophe
Shrinkage
rescue
Growth

7


Instrinsic properties of tubulin
Microtubule-Associated Proteins (MAPs) and Microtubule Inner Proteins (MIPs)
Structural MAPs & MIPs
Motor MAPs
+TIPs
Treadmilling
Dynamic instability
Regulation of Microtubule dynamics
Interphase
Mitosis
Balance between stabilisers and destabilisers
Amplification of dynamics

8

PLUS-END TRACKING PROTEINS (+TIPS)



Perez, et al 1999 Cell 96, 517-527.

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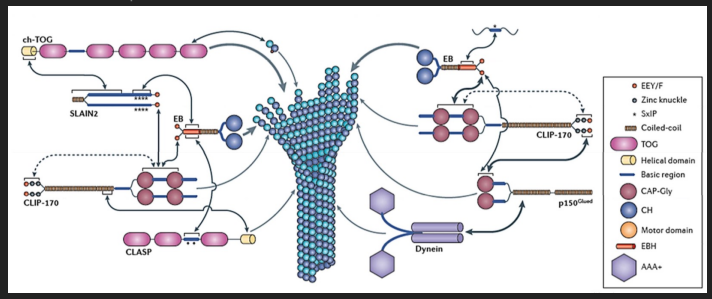
Tip-tracking of growing MTs mostly by treadmilling

The +TIPs : a complex network of molecular interactions



Akhmatova & Steinmetz, 2015 Nat Rev Mol Cell Biol, 16:711-26

EBs recognise the guanine nucleotide status of tubulin

EBs are central thanks to their

- EB Homology domain (EBH) → *Binding of **SxIP** and LxxPTP motifs*
- EEY/F C-ter sequence → *Recognition by **CAP-Gly** domains*

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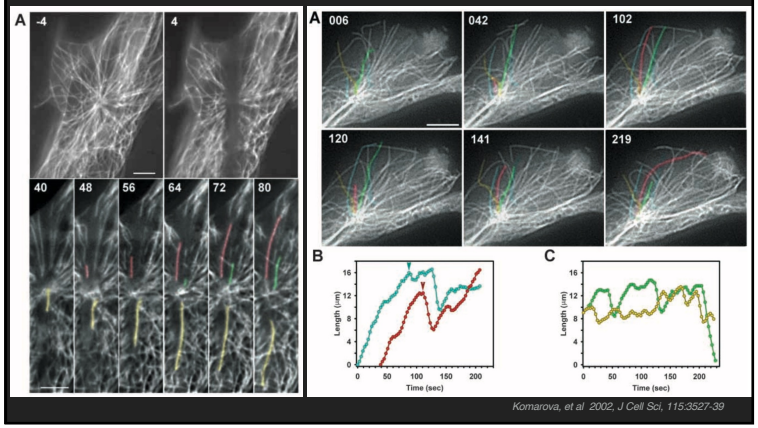
Functions of a few +TIPs

EBs

Promotion of persistant MT growth
Anti-catastrophe factor



Komarova, et al 2002, J Cell Sci, 115:3527-39

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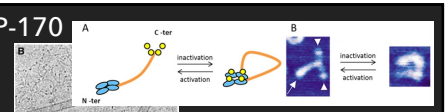
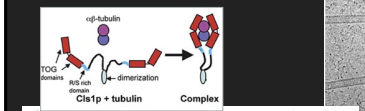
CLIP-170

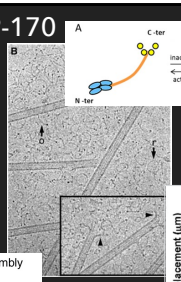
Rescue factors



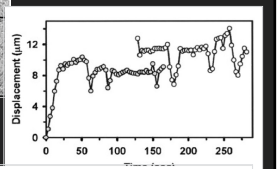
CLIP ΔH

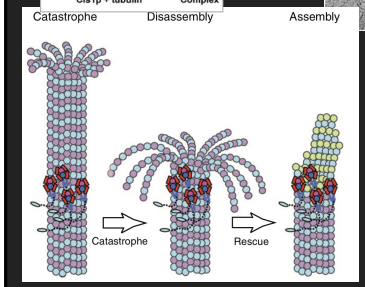
CLASPs

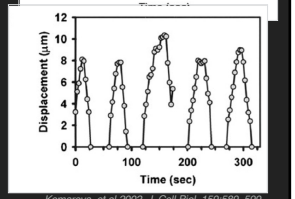




H2 + Tub







Al-Bassam J & Chang F. Trends Cell Biol. 2011:604–14.

Komarova, et al 2002. J. Cell Biol. 159:589–599.

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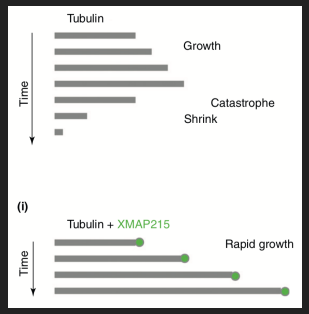
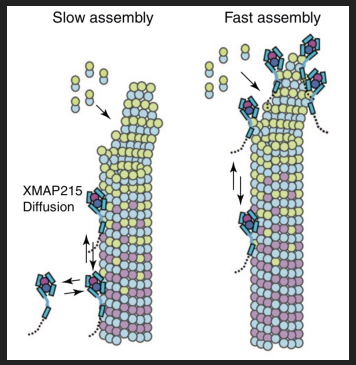
ch TOG / XMAP215

Processive polymerase





Al-Bassam J & Chang F. Trends Cell Biol. 2011:604–14.

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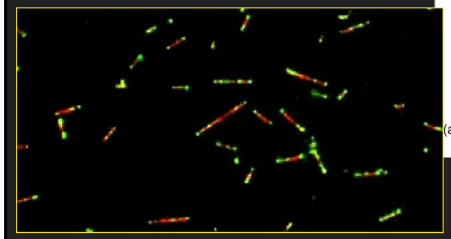
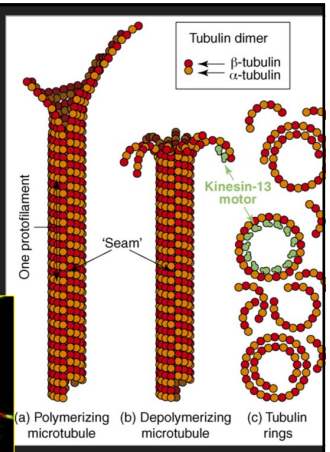
MCAK

Depolymerase

Catastrophin





Davis & Wordeman, Trends Cell Biol, 2007;17:37-42

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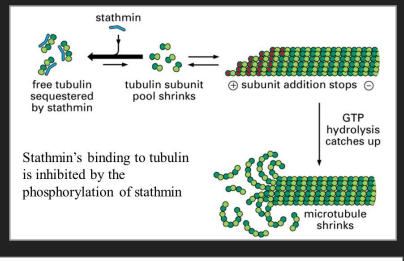
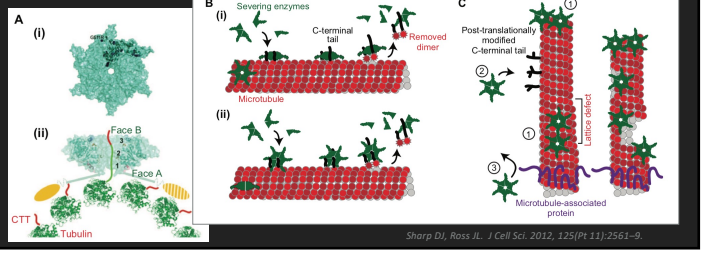
OTHER MT-DESTABILISERS

Stathmin /
Oncoprotein 18



Severing enzymes:
Spastin
Katanin
Fidgetin



Sharp DJ, Ross JL. J Cell Sci. 2012, 125(Pt 11):2561–9.

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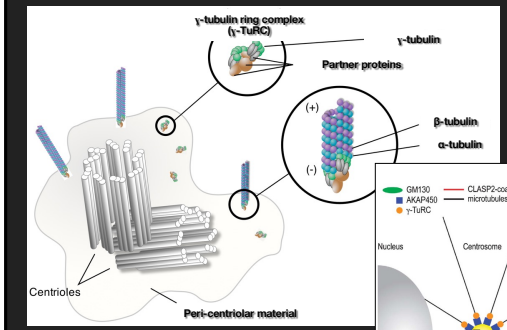
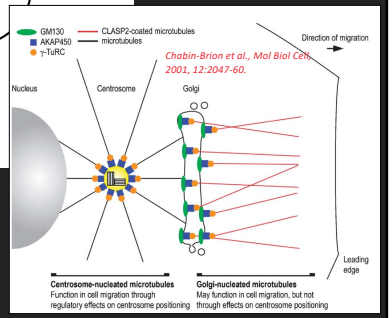
MT NUCLEATION AND MINUS-END ANCHORING



Poüs C, UE2 Flammarion Med Sci Tachdjian G Ed, 2012



Chabin-Brion et al., Mol Biol Cell, 2001, 12:2047-60.

Kodani & Sütterlin, EMBO J,2009,28:995-6

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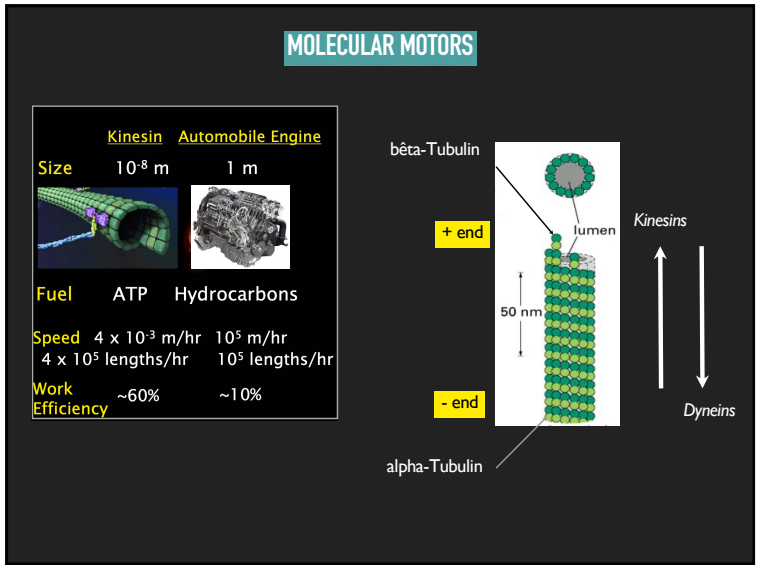
MOLECULAR MOTORS
Kinesin
Automobile Engine
Size
10-8 m
1 m
Fuel
ATP
Hydrocarbons
Speed
4 x 10-3 m/hr
10⁵ m/hr
4 x 10⁵ lengths/hr
10⁵ lengths/hr
Work Efficiency
~60%
~10%
bêta-Tubulin
+ end
lumen
50 nm
- end
alpha-Tubulin
Kinesins
Dyneins

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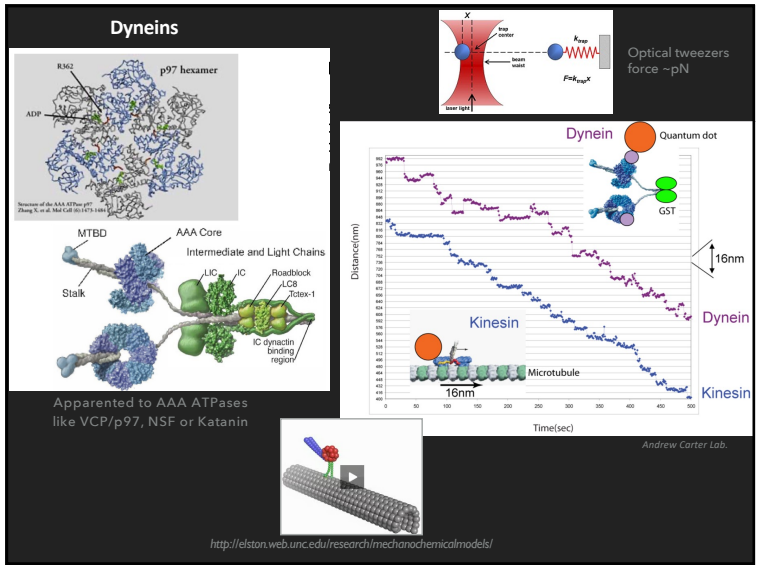
Dyneins
p97 hexamer
R362
ADP
MTBD
AAA Core
Intermediate and Light Chains
Stalk
LIC
IC
Roadblock
LC8
Tctex-1
IC dynactin binding region
Apparented to AAA ATPases like VCP/p97, NSF or Katanin
Optical tweezers force ~pN
Dynein
Quantum dot
GST
16nm
Kinesin
Microtubule
Distance(nm)
Time(sec)
Andrew Carter Lab.
http://elston.web.unc.edu/research/mechanochemicalmodels/

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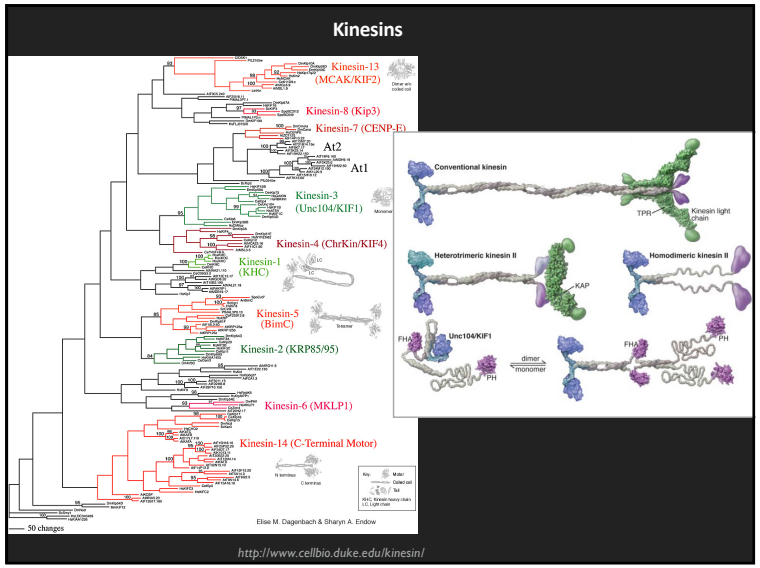
Kinesins
Kinesin-13 (MCAK/KIF2)
Kinesin-8 (Kip3)
Kinesin-7 (CENP-E)
At2
At1
Kinesin-3 (Unc104/KIF1)
Kinesin-4 (ChrKin/KIF4)
Kinesin-1 (KHC)
Kinesin-5 (BimC)
Kinesin-2 (KRP85/95)
Kinesin-6 (MKLP1)
Kinesin-14 (C-Terminal Motor)
Elise M. Dagenbach & Sharyn A. Endow
50 changes
Conventional kinesin
TPR
Kinesin light chain
Heterotrimeric kinesin II
KAP
Homodimeric kinesin II
Unc104/KIF1
FHA
PH
dimer
monomer
http://www.cellbio.duke.edu/kinesin/

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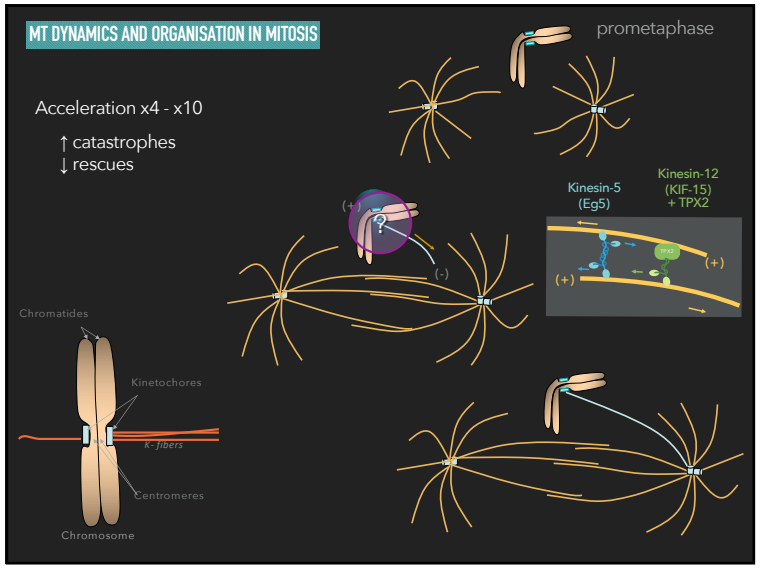
MT DYNAMICS AND ORGANISATION IN MITOSIS
prometaphase
Acceleration x4 - x10
↑ catastrophes
↓ rescues
Kinesin-5 (Eg5)
Kinesin-12 (KIF-15) + TPX2
Chromatides
Kinetochores
K-fibers
Centromeres
Chromosome

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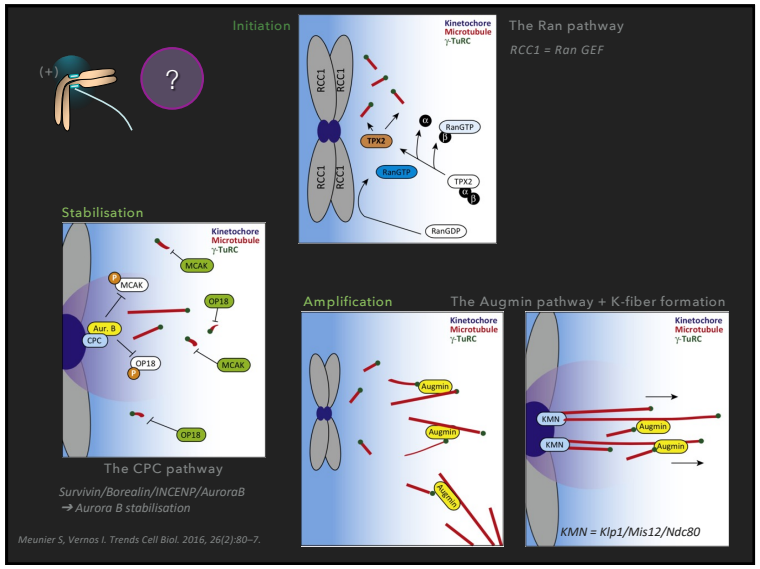
Initiation
The Ran pathway
RCC1 = Ran GEF
Kinetochore Microtubule γ-TuRC
RCC1
RanGTP
TPX2
RanGDP
Stabilisation
MCAK
OP18
Aur. B
CPC
The CPC pathway
Survivin/Borealin/INCENP/AuroraB
→ Aurora B stabilisation
Amplification
The Augmin pathway + K-fiber formation
Augmin
KMN
KMN = Klp1/Mis12/Ndc80
Meunier S, Vernos I. Trends Cell Biol. 2016, 26(2):80–7.

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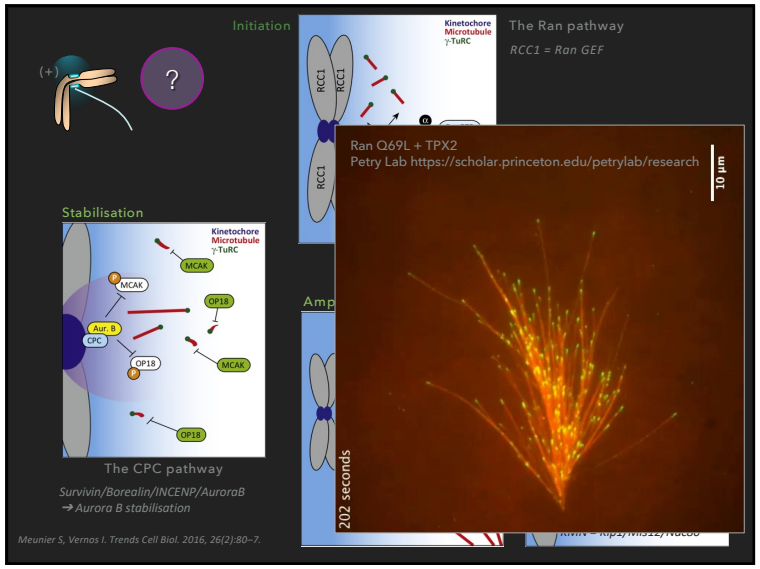
Initiation
The Ran pathway
RCC1 = Ran GEF
Ran Q69L + TPX2
Petry Lab https://scholar.princeton.edu/petrylab/research
10 µm
202 seconds
Stabilisation
The CPC pathway
Survivin/Borealin/INCENP/AuroraB
→ Aurora B stabilisation
Meunier S, Vernos I. Trends Cell Biol. 2016, 26(2):80–7.

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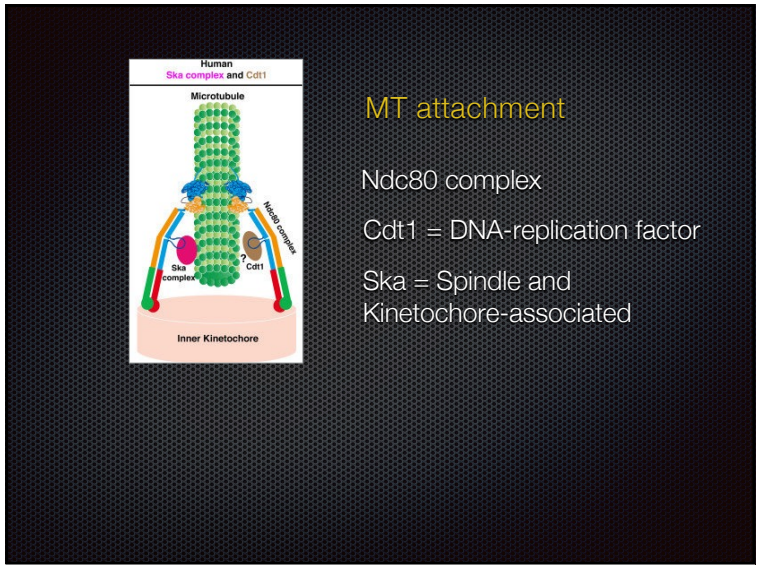
Human
Ska complex and Cdt1
Microtubule
Ndc80 complex
Ska complex
Cdt1
Inner Kinetochore
MT attachment
Ndc80 complex
Cdt1 = DNA-replication factor
Ska = Spindle and Kinetochore-associated

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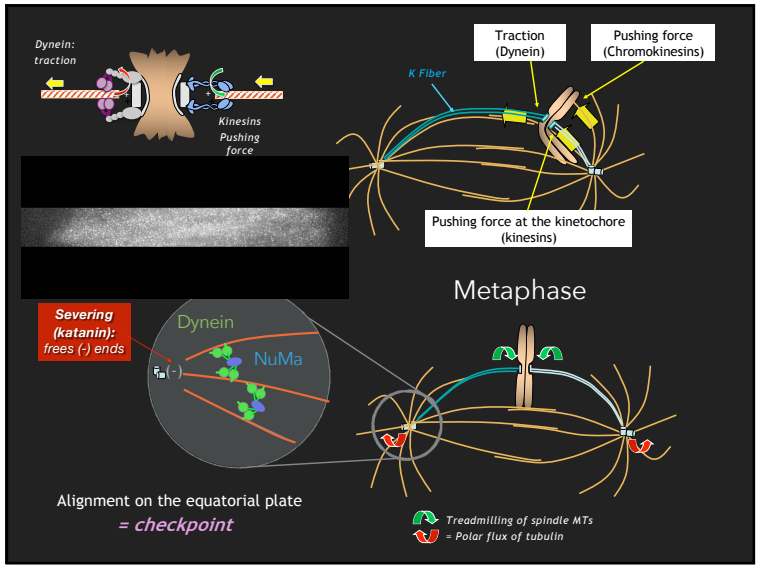
Dynein: traction
Kinesins Pushing force
Traction (Dynein)
Pushing force (Chromokinesins)
K Fiber
Pushing force at the kinetochore (kinesins)
Metaphase
Severing (katanin): frees (-) ends
Dynein
NuMa
(-)
Alignment on the equatorial plate
= checkpoint
Treadmilling of spindle MTs
= Polar flux of tubulin

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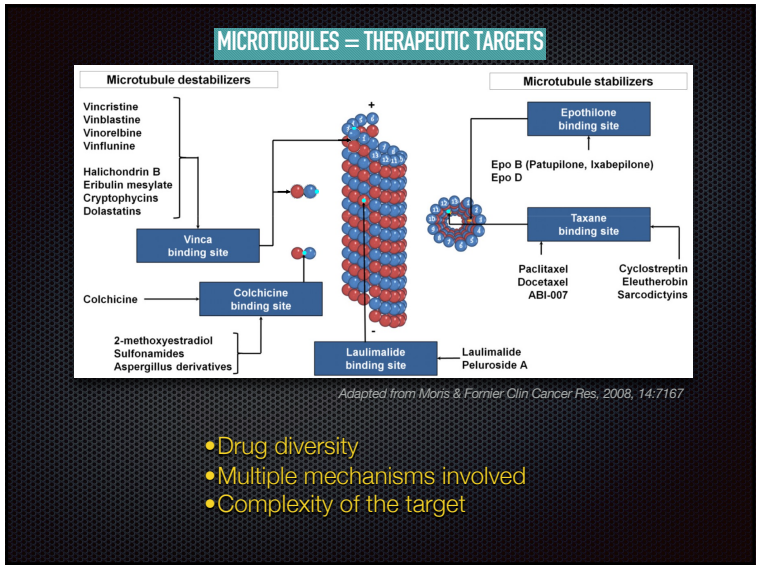
MICROTUBULES = THERAPEUTIC TARGETS
Microtubule destabilizers
Microtubule stabilizers
Vincristine
Vinblastine
Vinorelbine
Vinflunine
Halichondrin B
Eribulin mesylate
Cryptophycins
Dolastatins
Vinca binding site
Colchicine
Colchicine binding site
2-methoxyestradiol
Sulfonamides
Aspergillus derivatives
Laulimalide binding site
Laulimalide
Peluroside A
Epothilone binding site
Epo B (Patupilone, Ixabepilone)
Epo D
Taxane binding site
Paclitaxel
Docetaxel
ABI-007
Cyclostreptin
Eleutherobin
Sarcodictyins
Adapted from Moris & Fornier Clin Cancer Res, 2008, 14:7167
•Drug diversity
•Multiple mechanisms involved
•Complexity of the target

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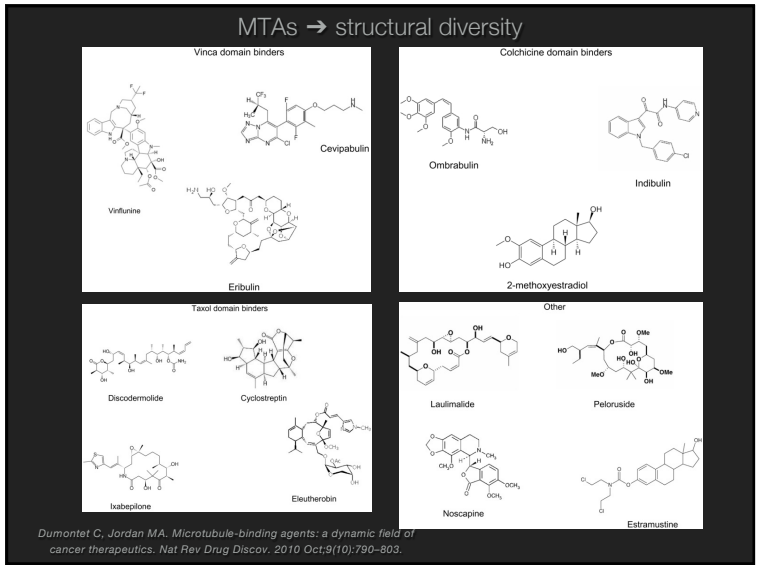
MTAs ➔ structural diversity
Vinca domain binders
Cevipabulin
Vinflunine
Eribulin
Colchicine domain binders
Ombrabulin
Indibulin
2-methoxyestradiol
Taxol domain binders
Discodermolide
Cyclostreptin
Ixabepilone
Eleutherobin
Other
Laulimalide
Peloruside
Noscapine
Estramustine
Dumontet C, Jordan MA. Microtubule-binding agents: a dynamic field of cancer therapeutics. Nat Rev Drug Discov. 2010 Oct;9(10):790–803.

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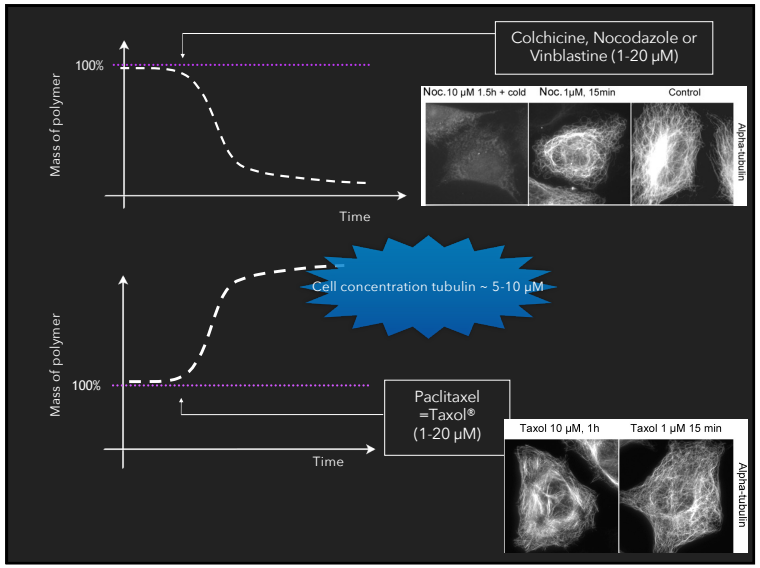
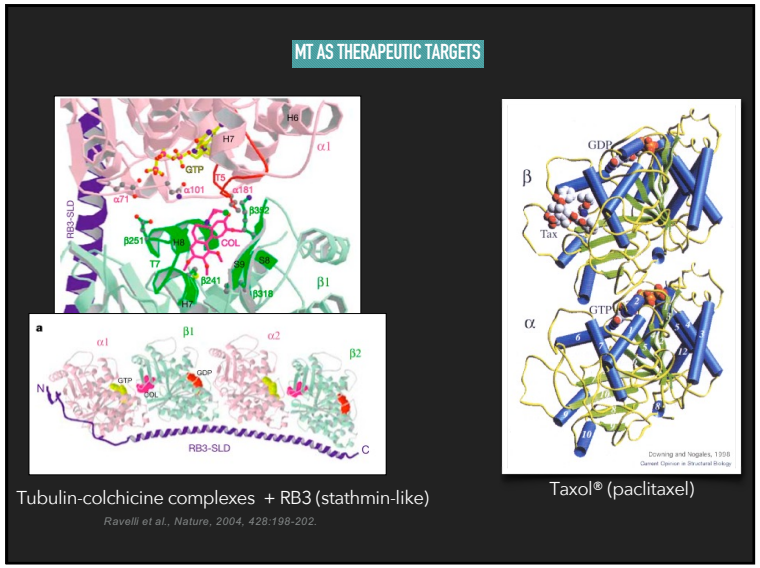

Colchicine, Nocodazole or Vinblastine (1-20 µM)
100%
Mass of polymer
Time
Noc.10 µM 1.5h + cold
Noc.1µM, 15min
Control
Alpha-tubulin
Cell concentration tubulin ~ 5-10 µM
Paclitaxel =Taxol® (1-20 µM)
Taxol 10 µM, 1h
Taxol 1 µM 15 min

39


MT AS THERAPEUTIC TARGETS
RB3-SLD
GTP
GDP
COL
Tax
Tubulin-colchicine complexes + RB3 (stathmin-like)
Ravelli et al., Nature, 2004, 428:198-202.
Taxol® (paclitaxel)

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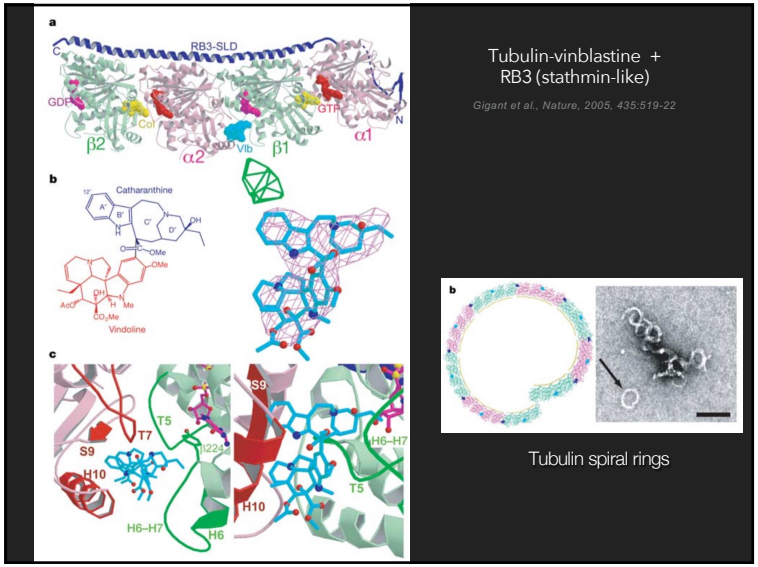

Tubulin-vinblastine + RB3 (stathmin-like)

Gigant et al., Nature, 2005, 435:519-22

Tubulin spiral rings

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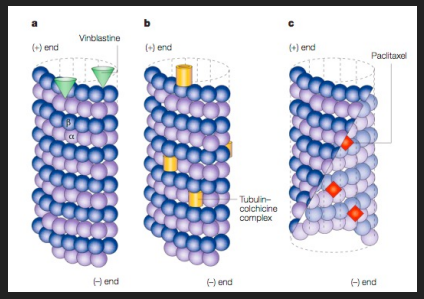

1 to 2 molecules of vinblastine by MT → *Reduction by 50% of treadmilling and dynamic instability*

Jordan & Wilson, Nat. Rev. Cancer, 2004, 4:253-65.

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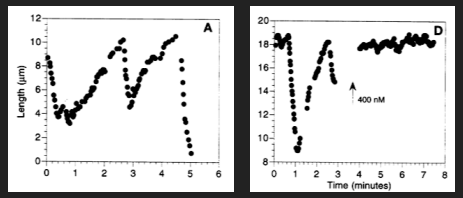
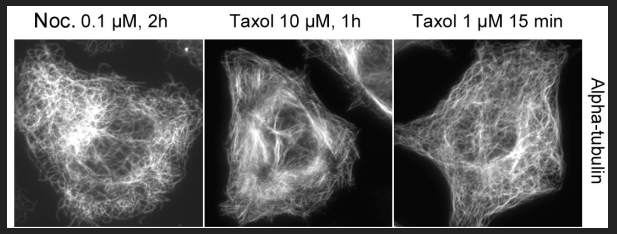
Low doses of anti-polymerizing agents inhibit MT dynamics



Vasquez et al., Mol. Biol. Cell, 1997, 8:973-85.



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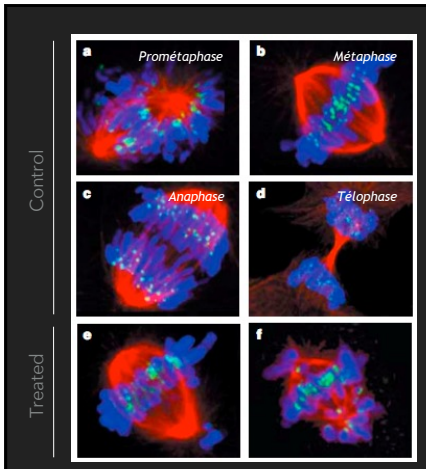

Blockade at the transition from metaphase to anaphase

Consequences:

- *aneuploïdy*
- *apoptosis*
- *….others ?*

microtubules
kinetochores
DNA

Jordan & Wilson, Nat. Rev. Cancer, 2004, 4:253-65.

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MITOSIS = THERAPEUTIC TARGET ?

nature REVIEWS CLINICAL ONCOLOGY

PERSPECTIVES

OPINION

Mitosis is not a key target of microtubule agents in patient tumors

Edina Komlodi-Pasztor, Dan Sackett, Julia Wilkerson and Tito Fojo

Komlodi-Pasztor, E. *et al. Nat. Rev. Clin. Oncol.* **8**, 244–250 (2011);

Table 1 | Mitotic index in patient samples

Tumor type	Mitotic index (mitosis/ 1,000 tumor cells)
Breast cancer	5 (mean)[6] 1.2 (mean)[7] 1–5 (median)[8] 2 (mean)[9] 5.8 (mean)[10]
Ovary granulosa	0.2 (median)[11]
Uterine cervical	9 (mean)[12]
Prostate cancer	15 (mean)[13]

Edina, Komlodi-Pasztor, et al. (2011) (Not) too early to say. "no targeting of mitosis!" Nat. Rev. Clin. Oncol. doi:10.1038/nrclinonc.2010.228-c2

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Other potential targets ?

MT-dependent cellular functions

+

Interphase cells

Cell migration

Neo-angiogenesis

Autophagy

Traffic

Apoptosis

Signaling

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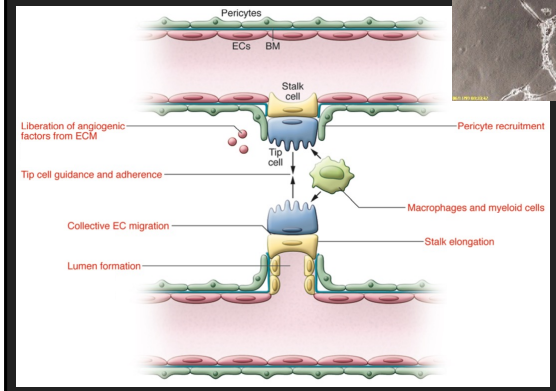
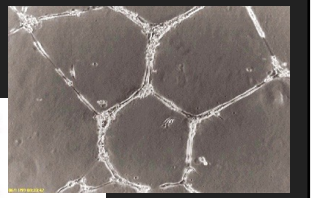
Neo-angiogenesis





Tube formation

Proliferation
Migration
Junction formation

Welti et al., J. Clin. Invest. 2013, 123:3190-3200

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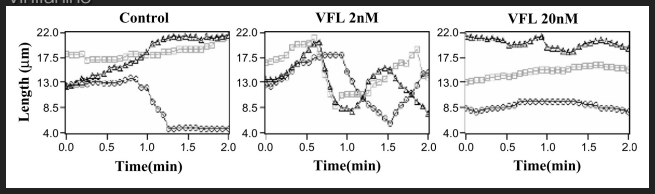
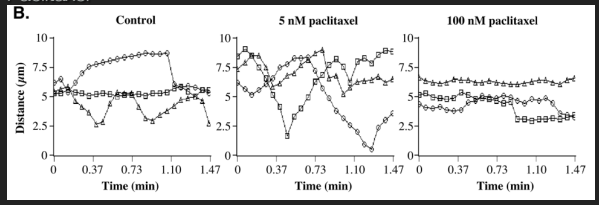
Endothelial Cells

Vinflunine



Pourroy et al., Cancer Res, 2006, 66:3256-63

Paclitaxel



Pasquier et al., Cancer Res, 2005, 65:2433-40

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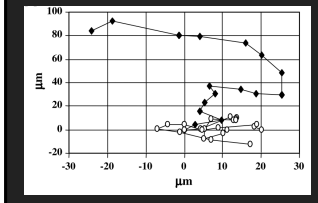
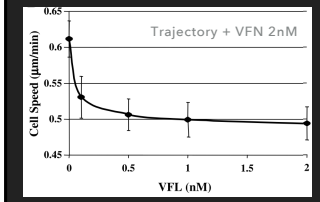
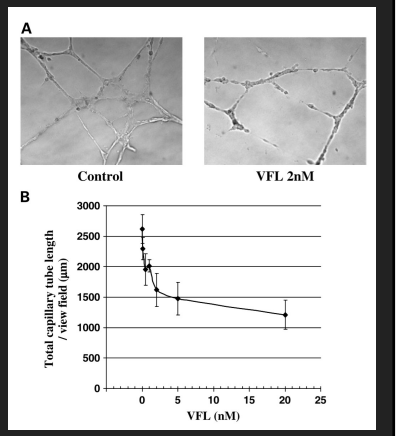
Anti-migratory effects

Endothelial cells





Pourroy B et al., Cancer Res., 2006, 66:3256-63



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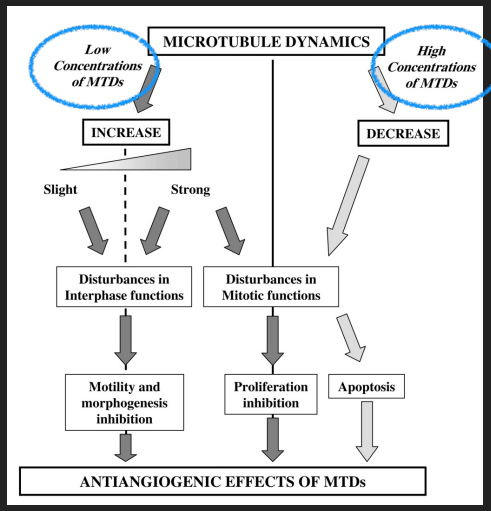
nM



> 10 nM

Pourroy et al., Cancer Res, 2006, 66:3256-63

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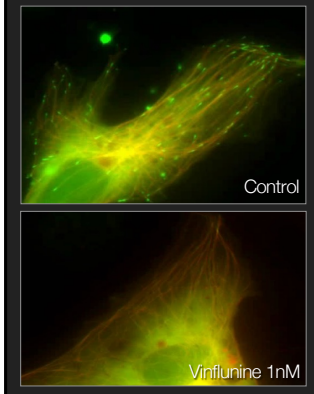
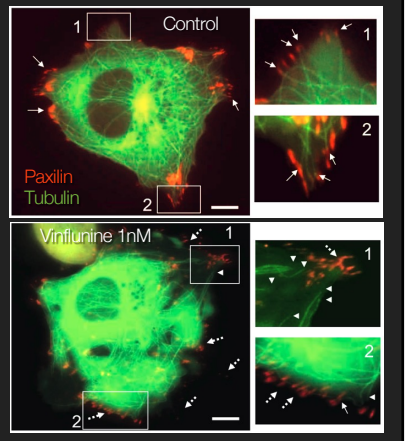
EB loss



Honoré S. Mol. Cancer Ther, 2008, 7:2080-9

Defect in focal adhesion targeting



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- Mechanisms of acquired and intrinsic drug resistance

Effectiveness variability depending on tissues and organs:

- *Taxol effective on ovarian, lung or breast cancers not effective on other carcinomas (kidney, colon…)*
- *Vinca alcaloids : heamatological cancers, not on solid tumours*

Cell resistance

- *Extracellular efflux by ABC transporters (PGP, gene MDR1)*
- *Level of expression of regulatory proteins*
- *Alteration of PTMs*
- *Modification of the tubulin isotope profiles (β III tubulin)*
- *Mutations on α-, β-tubulin, Stathmin, MAP4*
- *Other…*

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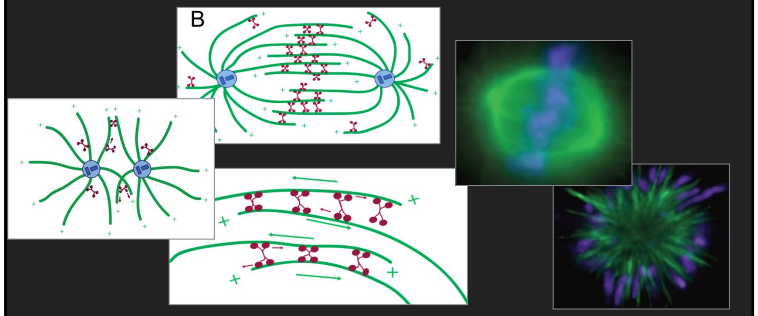
- New paradigms for therapeutic targeting

a) Increase the molecular diversity of tubulin-interacting drugs ➔ wider repertoire of active molecules

b) Target other components : e.g. Kinesin-5 (Eg5) inhibition ➔mono-astral spindle



Valentine et al., BMC Cell Division, 2006, 1:31. *Mayer et al., Science. 1999 Oct 29;286(5441):971-4*

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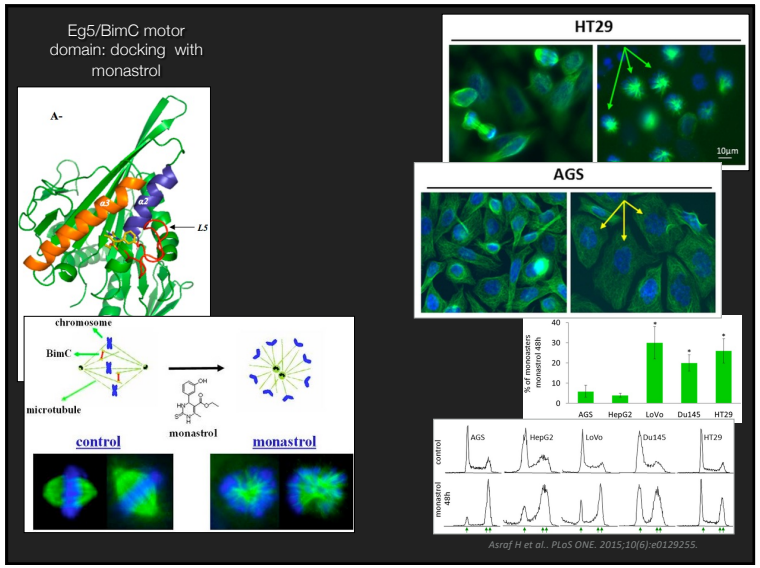

Asraf H et al.. PLoS ONE. 2015;10(6):e0129255.

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CONCLUSIONS

- High dynamicity required for MT physiological functions.
- Complexity of the regulations.
- Therapeutic target: better understand the physiology and the regulations to apprehend drug effects.
- Importance of overcoming drug resistance. Identify mechanisms of intrinsic and acquired resistance.
- Alternative targeting.
- Emerging concepts of regulation.

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