The microtubule cytoskeleton: an old validated target for novel anti-tumor drugs with new mechanisms of action

Institute for **Advanced Biosciences**

CENTRE DE RECHERCHE UGA – INSERM U 1209 – CNRS UMR 5309

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Team “Cytoskeleton Dynamics and Nuclear Functions”

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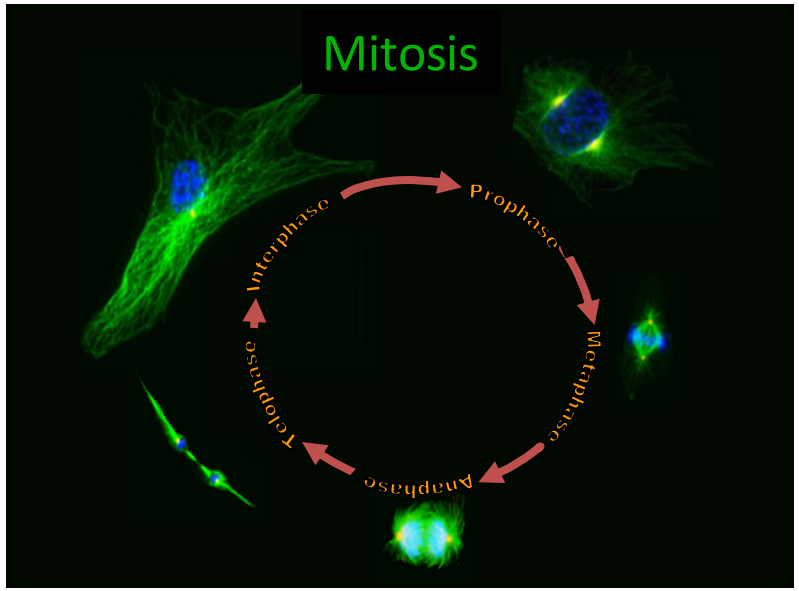
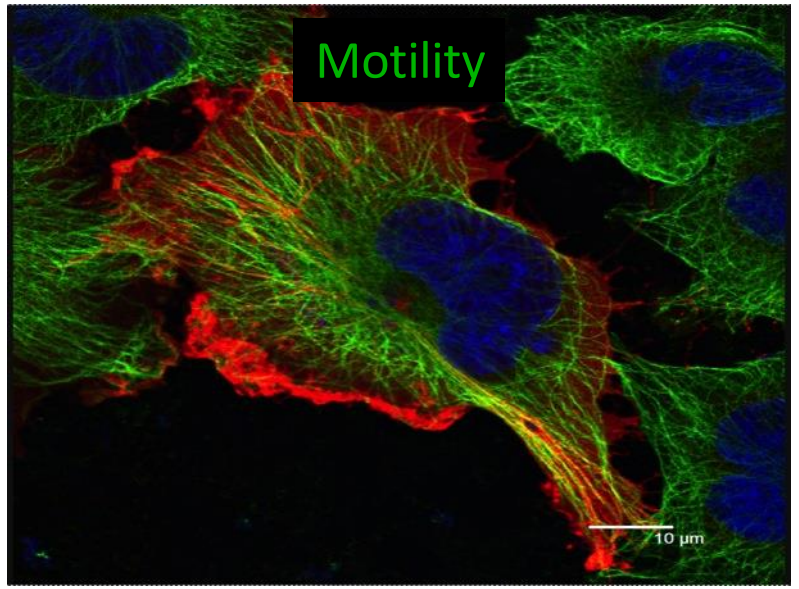
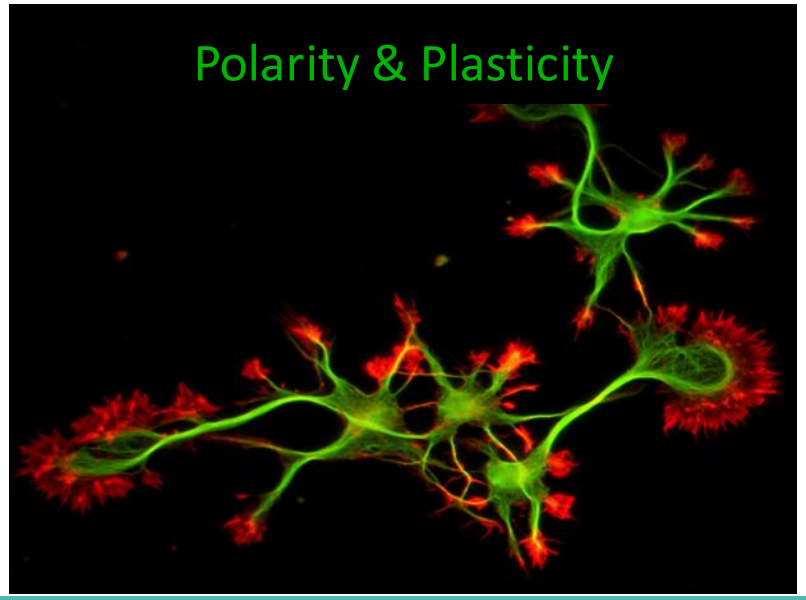
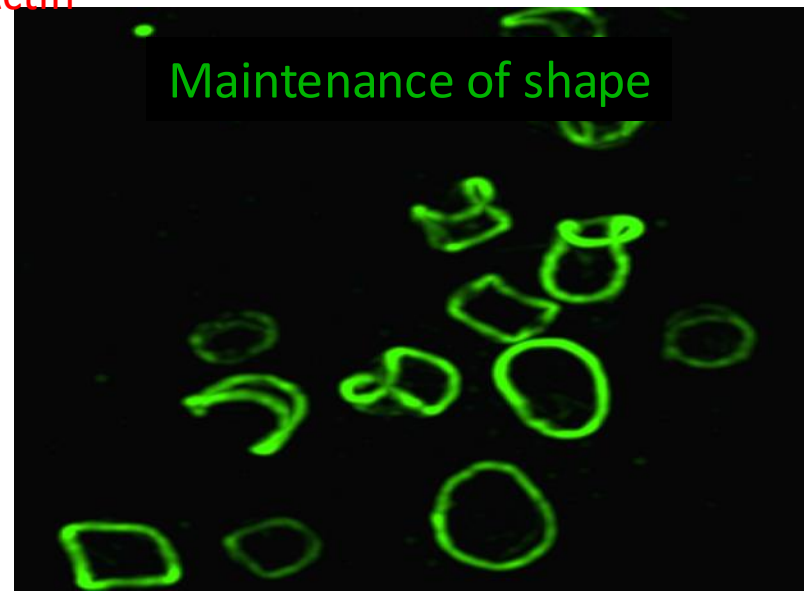
Microtubules and actin microfilaments are involved in key cell functions





Tubulin Actin





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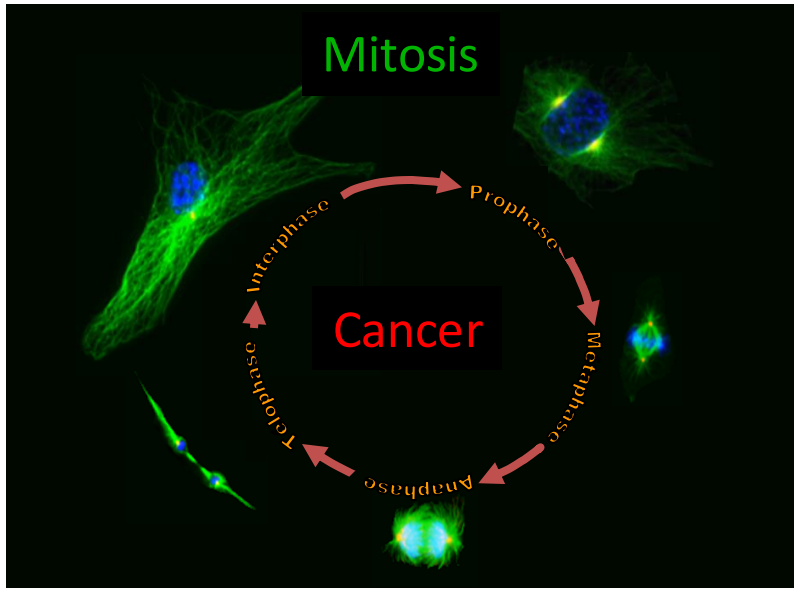
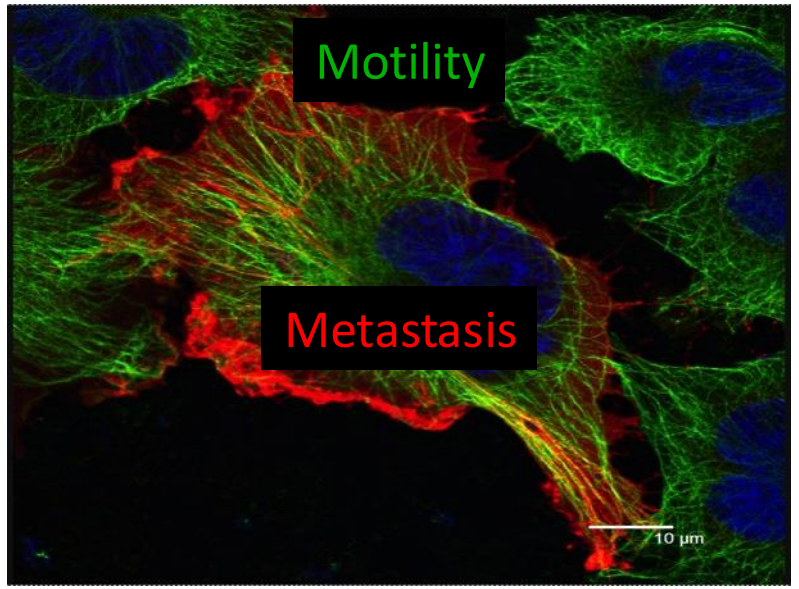
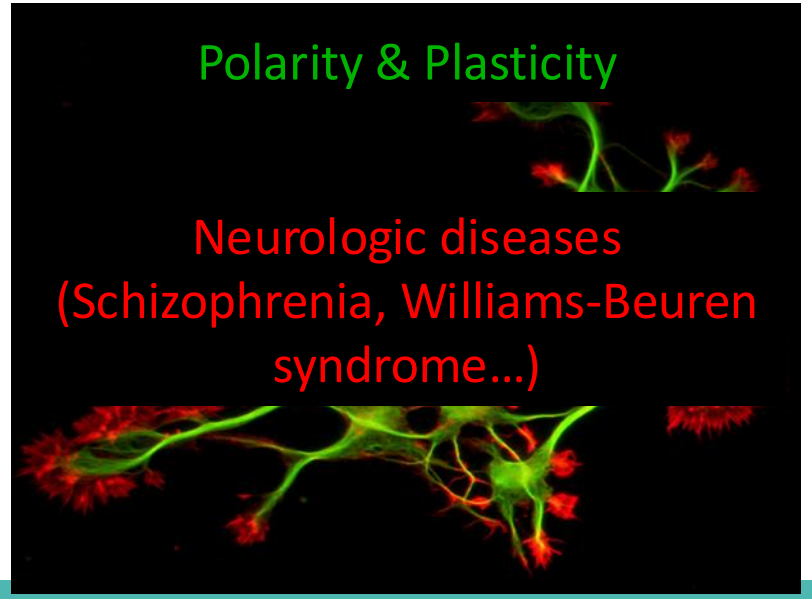
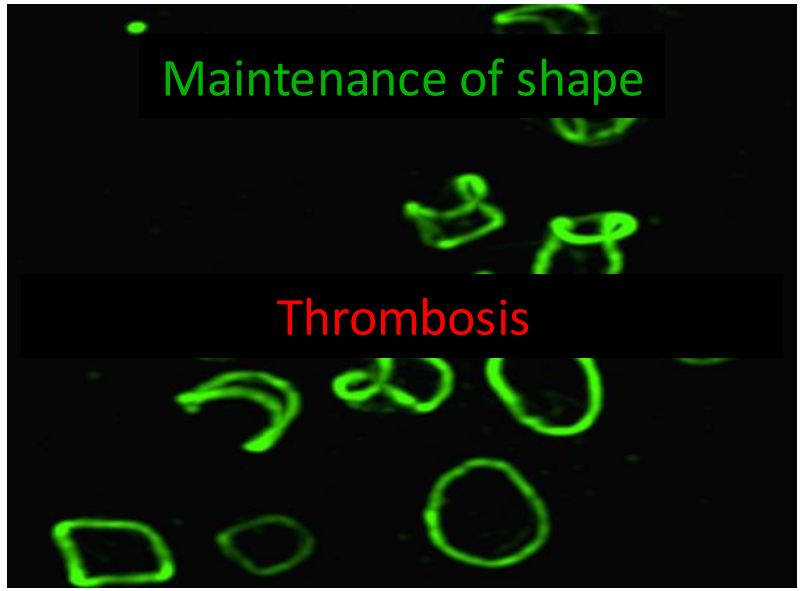
Perturbation of the regulation of the cytoskeleton can lead to diseases









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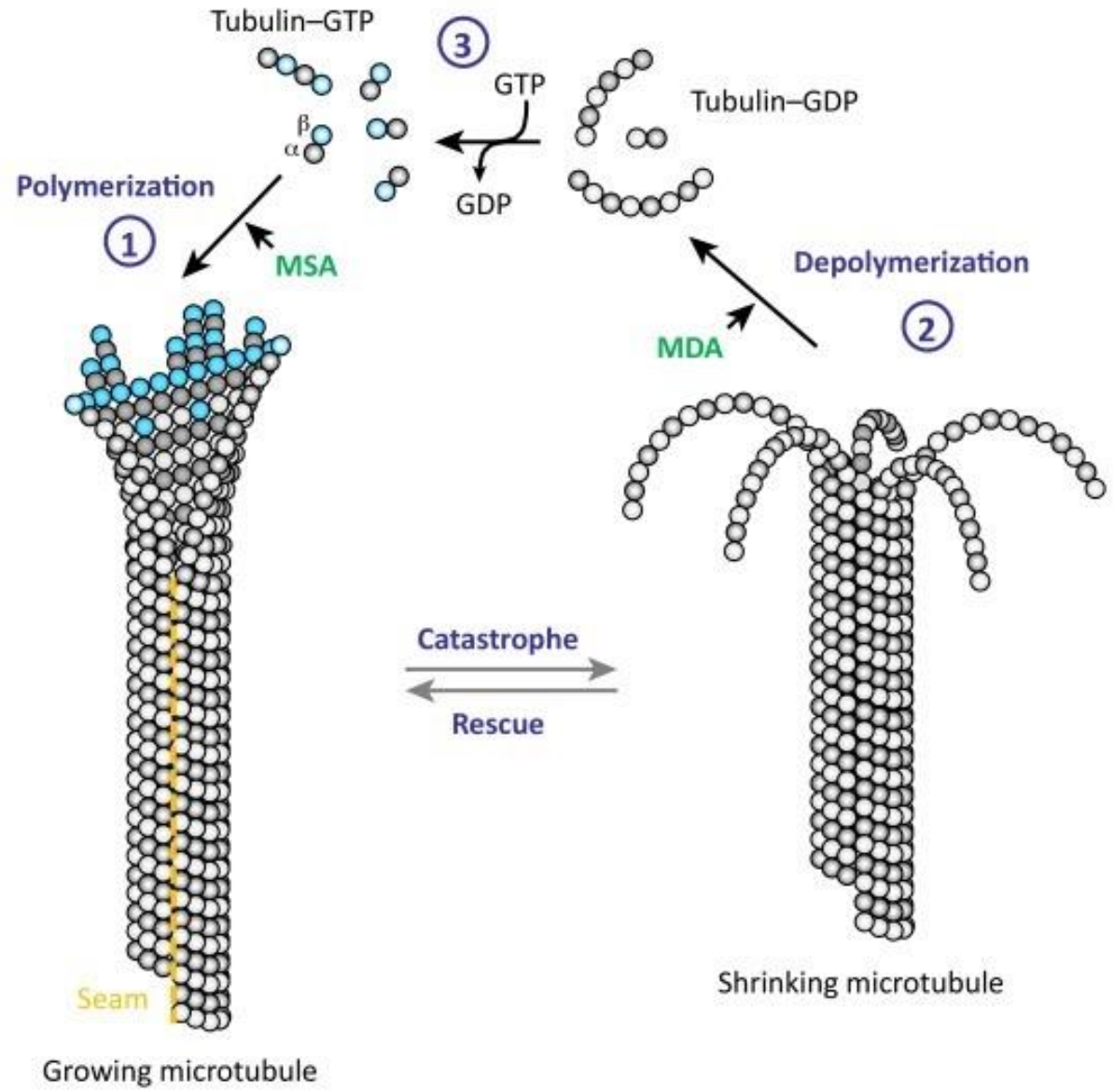
Microtubules are dynamic structures composed of α/β tubulin dimers

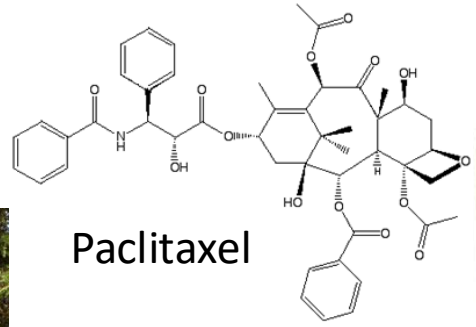


Microtubule dynamics is controlled :

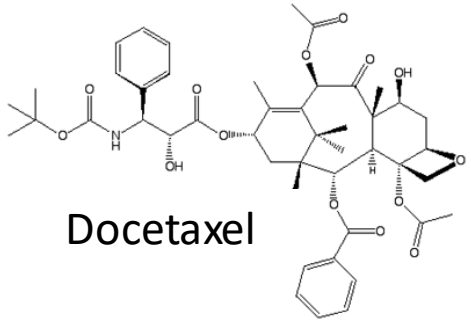
- By associated proteins

- By post-translational modifications

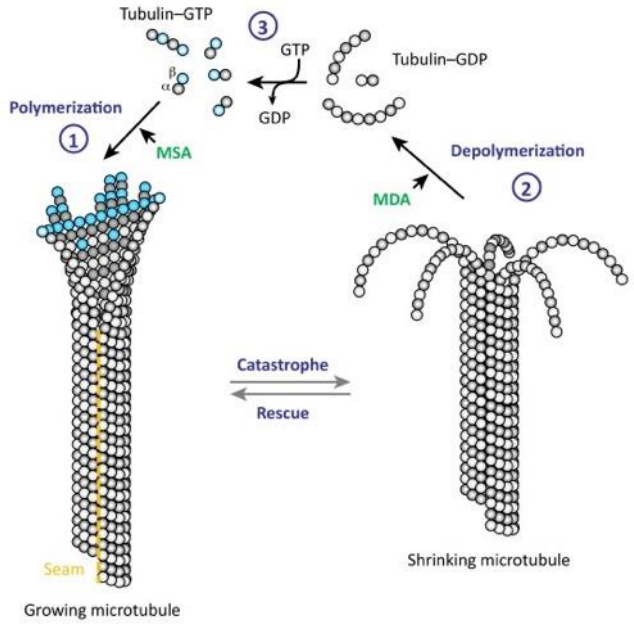
"Mitotic" poisons : major anti-tumor drugs



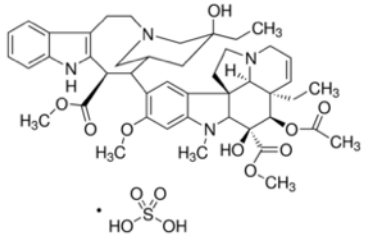
Paclitaxel



Docetaxel

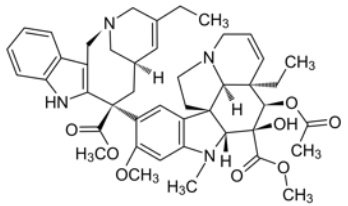


Microtubule Depolymerizing Agents



Vinblastine

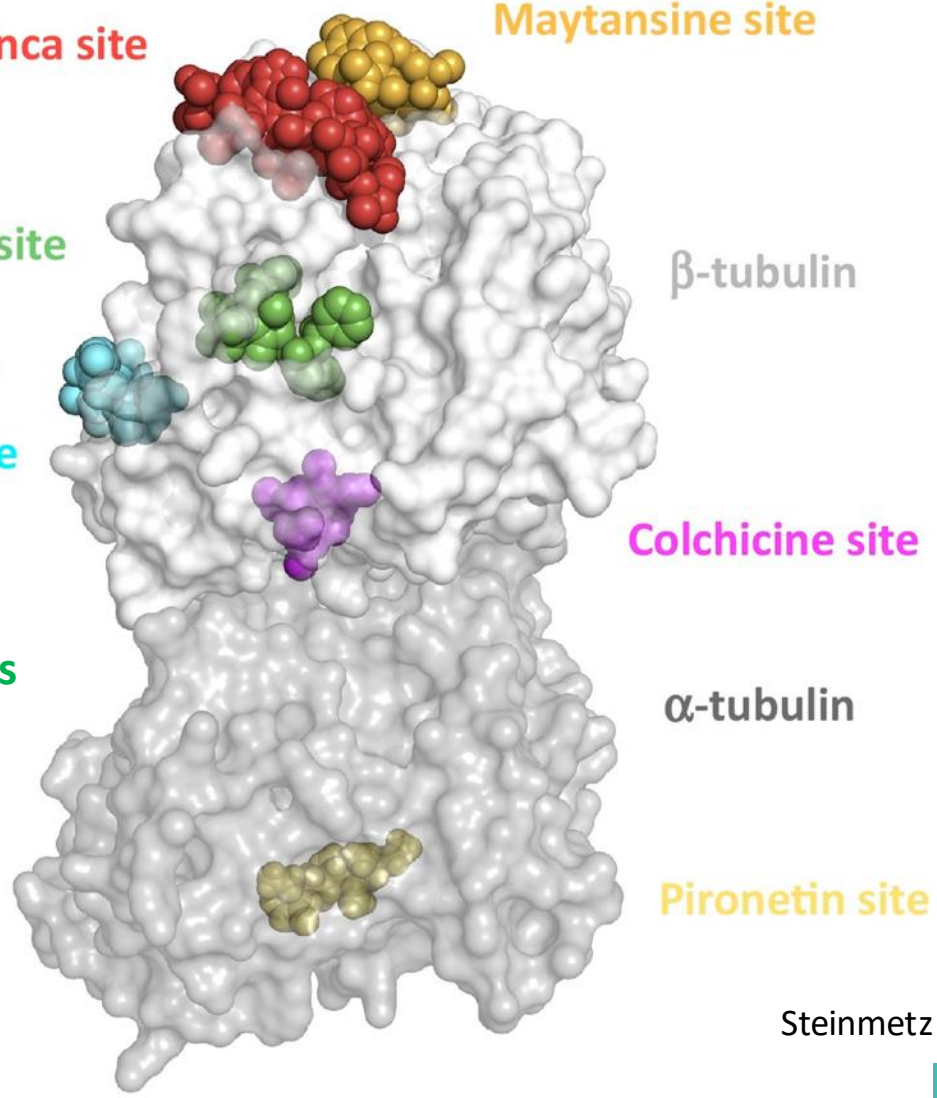
Vinorelbine



Microtubule Stabilizing Agents

Microtubule-Targeting Agents Binding Sites on Tubulin

Microtubule Depolymerizing Agents



Microtubule Stabilizing Agents

Steinmetz & Prota, 2018

Potent anticancer agents

Leukemia

Solid tumors

- Breast
- Ovary
- Brain
- Lung
- Etc.

Problems

- Low solubility in water
- Undesirable side effects (hair loss, nausea, lowered blood cell counts, headache, stomach pain, peripheral neurotoxicity, etc.)
- Resistances

Our aims

- ✓ To identify crucial microtubules actors deregulated in cancers and other diseases
- ✓ To find pharmacological agents that target these proteins
- ✓ To test their therapeutic efficacy, on *in vit*ro cell models, on animal models and on *ex vivo* human samples.

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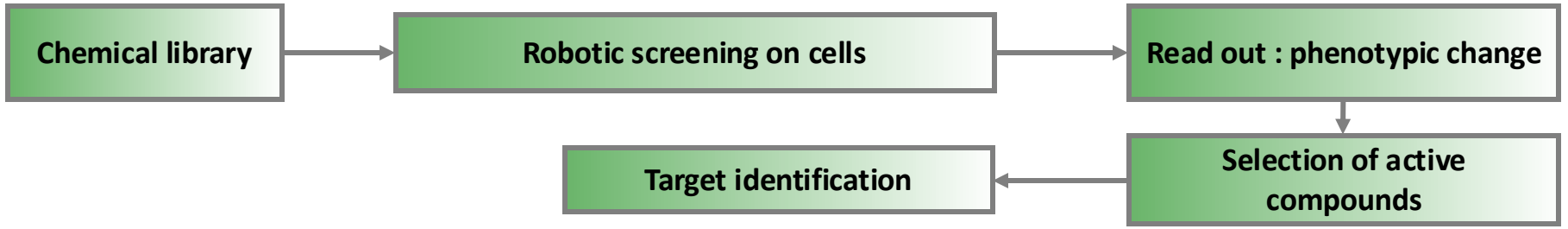
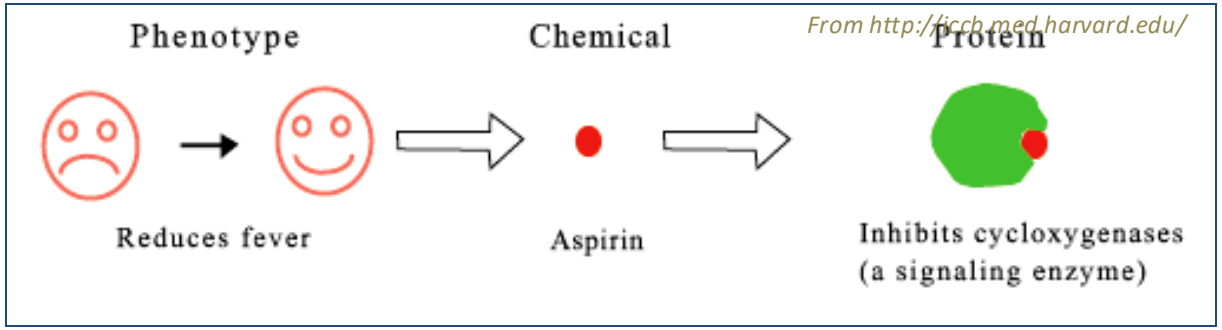
Methodology and specific tools:
Specific small molecules discovered by phenotypic screening

Small molecules that diffuse rapidly into the cytoplasm are valuable probes to study dynamic biological processes





From http://icch.med.harvard.edu/

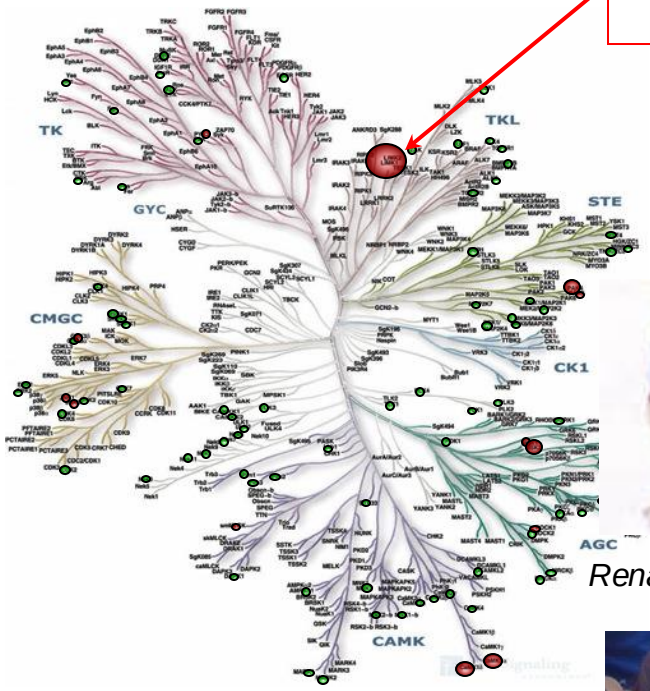
Not different to classical pharmacological strategies…
…but accelerated thanks to miniaturization, use of robots, access to chemical libraries, large data management and analysis

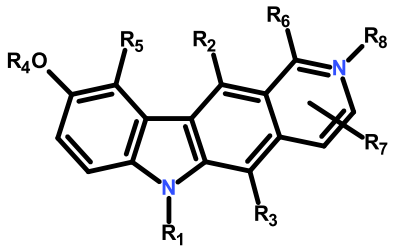
This approach, when successful, allows to simultaneously find new drugs and new effectors

Discovery of Pyr1, a selective LIMK Inhibitor

30,080 molecules → Cell-based assay for microtubule stability → increase of Detyr-tubulin → Selection of pyridocarbazolone → (3 years) → LIMK identification

CMBA
plateforme de Criblage pour des Molécules BioActives





Pyr1

Inhibition of only one kinase out of 110 kinases tested

Renaud Prudent

Chloé Prunier

Published OnlineFirst on July 3, 2012; DOI:10.1158/0008-5472.CAN-11-3342

Therapeutics, Targets, and Chemical Biology

Cancer Research

Pharmacological Inhibition of LIM Kinase Stabilizes Microtubules and Inhibits Neoplastic Growth

Renaud Prudent[1], Emilie Vassal-Stermann[2], Chi-Hung Nguyen[4,5,6], Catherine Pillet[2], Anne Martinez[1], Chloé Prunier[1], Caroline Barette[2], Emmanuelle Soleilhac[2], Odile Filhol[3], Anne Beghin[8], Glaucio Valdameri[9], Stéphane Honoré[7], Samia Aci-Sèche[2], David Grierson[4,5], Juliana Antonipillai[11], Rong Li[11], Attilio Di Pietro[9], Charles Dumontet[10], Diane Braguer[7], Jean-Claude Florent[4,5], Stefan Knapp[13], Ora Bernard[11,12], and Laurence Lafanechère[1,2]

Published OnlineFirst May 23, 2016; DOI: 10.1158/0008-5472.CAN-15-1864

Therapeutics, Targets, and Chemical Biology

Cancer Research

LIM Kinase Inhibitor Pyr1 Reduces the Growth and Metastatic Load of Breast Cancers

Chloé Prunier[1], Véronique Josserand[2], Julien Vollaire[2], Evelyne Beerling[3], Christos Petropoulos[4], Olivier Destaing[4], Christopher Montemagno[1], Amandine Hurbin[2], Renaud Prudent[1], Leanne de Koning[5], Reuben Kapur[6], Pascale A. Cohen[7], Corinne Albiges-Rizo[4], Jean-Luc Coll[2], Jacco van Rheenen[3], Marc Billaud[1], and Laurence Lafanechère[1]

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Conclusions #1

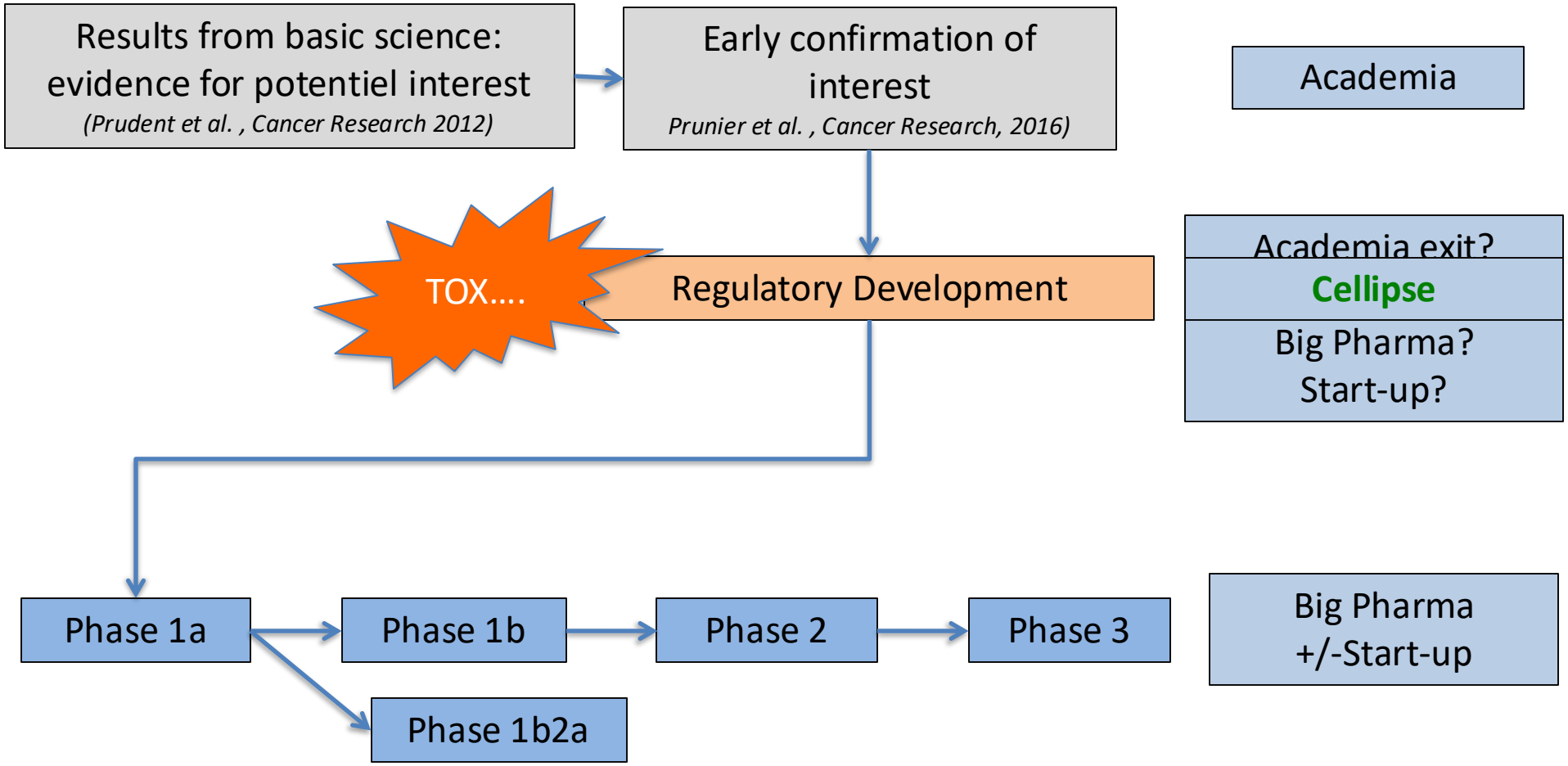
- Pyr1 is a “first in class” LIMK inhibitor, showing efficacy on mice tumor models

- It targets the cytoskeleton, including microtubules, with a novel mechanism of action

- We have evidence that it is efficient on Taxol® resistant cancers

- Although Pyr1 does not prevent metastasis establishment, we have evidence that it is efficient on the growth of secondary tumors

Drug development : Role of the different actors



- Synthesis and chemical optimization of LIMK inhibitors
- Pursuing GLP compliant preclinical development

Fondation of Cellipse SAS

Cellipse
Cell to drug discovery

3 co-founders with complementary skills :

- ✓ **L. Lafanechère**, PhD Biology
  Expert in microtubule dynamics and cancer
  President of Scientific Advisory Board
- ✓ **R. Prudent**, PhD Chemistry
  Chief Operating Officer (=manager) of CELLIPSE

Start-up is not a lab, other skills needed : business, fund raising, patent negociation, etc.

- ✓ **F. Paublant** Business (ESSEC, M.I.T., IPSEN, Boston Consulting Group)
  Chief Executive Officer (=boss)

Scientific Advisors :

- Pr Jean-Yves Blay – Medical Director CLB Lyon, Hon. President EORTC
- Dr Marc Billaud - IAB
- Pr Reuben Kapur – Indiana U. Herman B Wells Center
- PR Yangbo Feng, Scripps Institute, USA

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Drug development : Role of the different actors

Results from basic science: evidence for potentiel interest
(Prudent et al. , Cancer Research 2012)

Early confirmation of interest
Prunier et al. , Cancer Research, 2016)

Academia

Cellipse

TOX….

Regulatory Development

Phase 1a

Phase 1b

Phase 2

Phase 3

Phase 1b2a

Big Pharma
+/-Start-up

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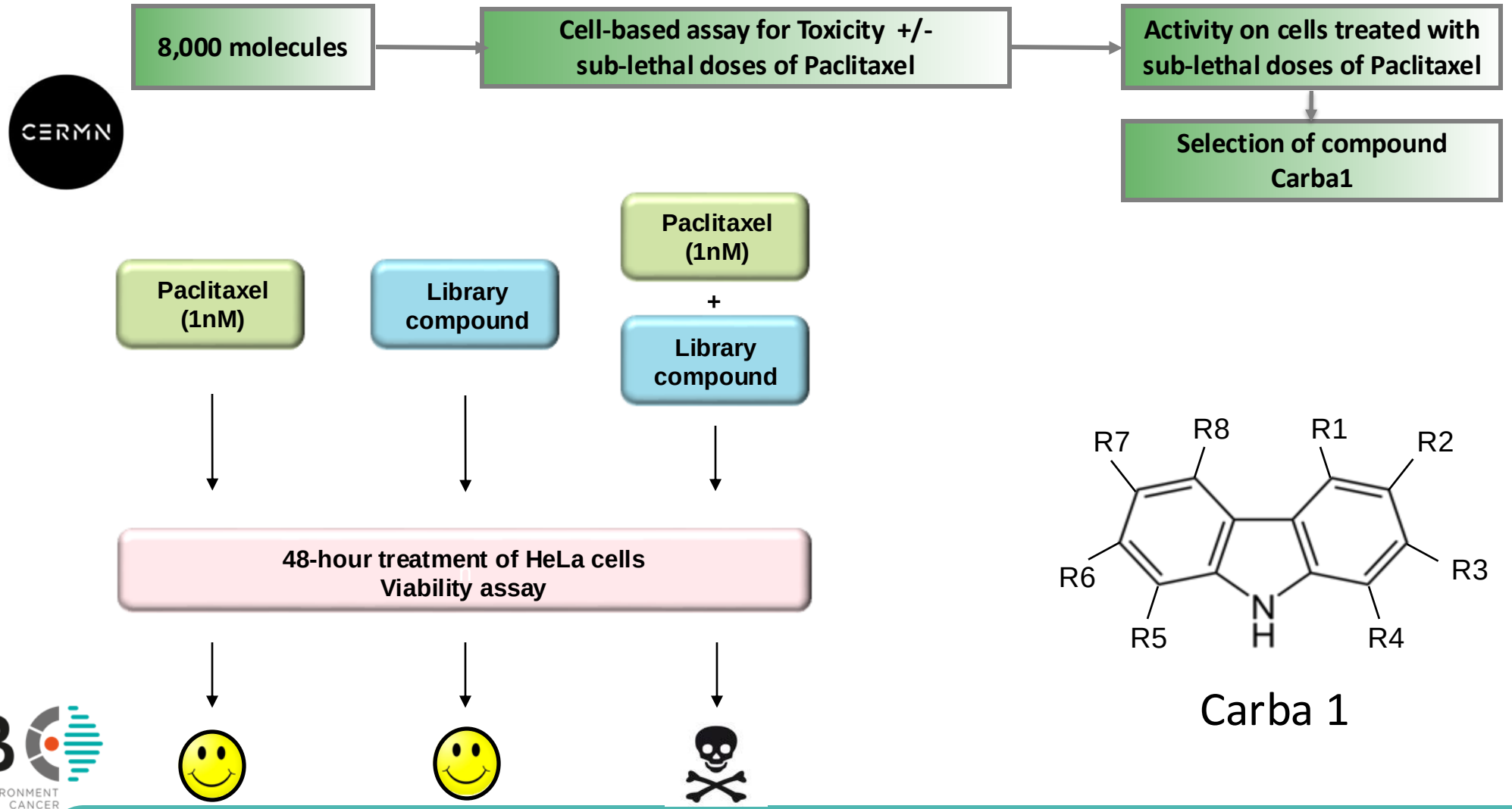
Search for compounds that sensitize cells to non lethal doses of paclitaxel

Why?

Lowering PTX therapeutic doses could avoid the occurrence of resistances and reduce undesirable side-effects such as neurotoxicity



Carba 1

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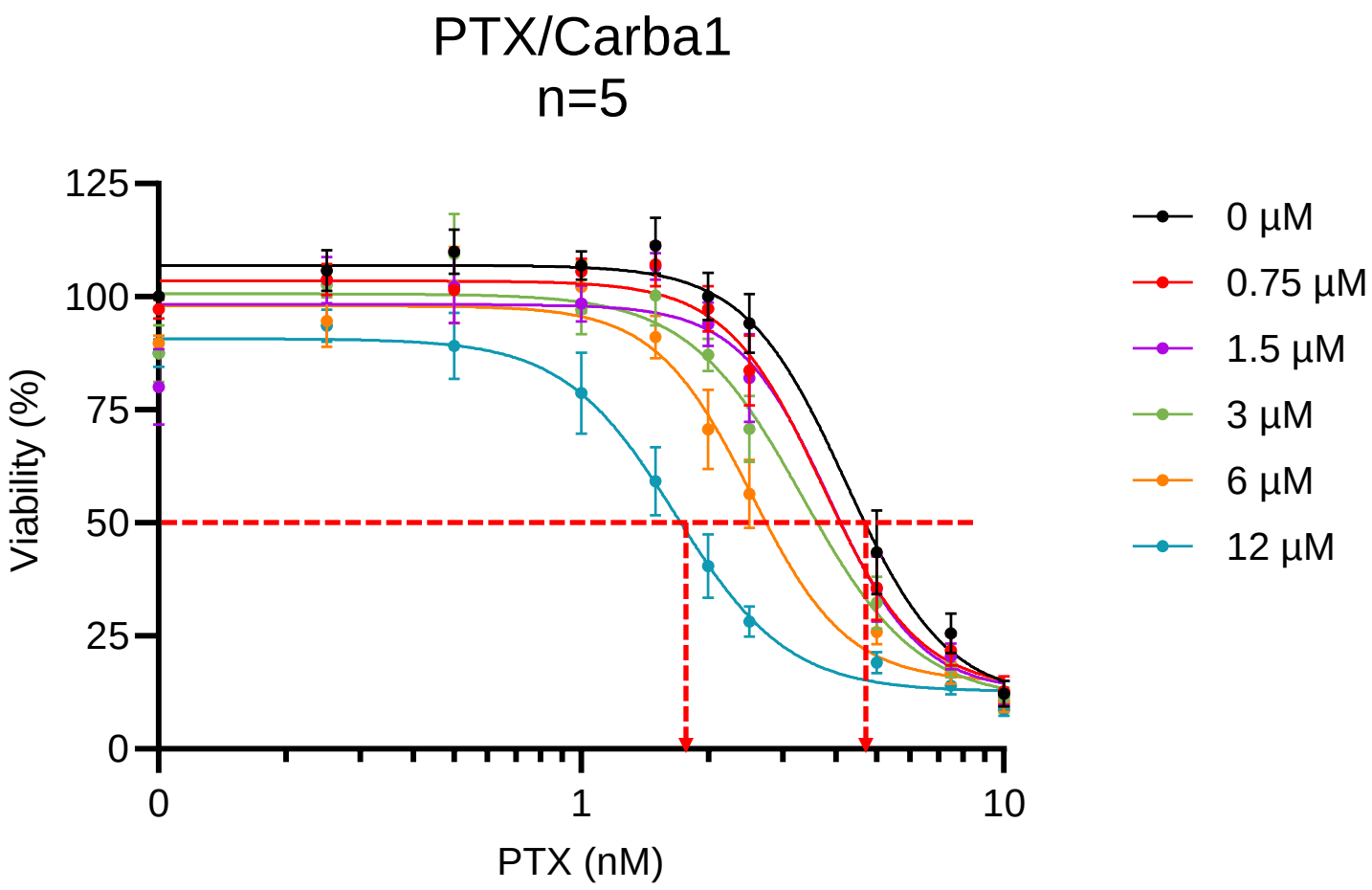
Carba1 Enhances the Cytotoxic Effect of PTX on Cell Viability



Without Carba1 PTX GI50 = 5 nM

With Carba1 12 µM PTX GI50 = 0.9 nM

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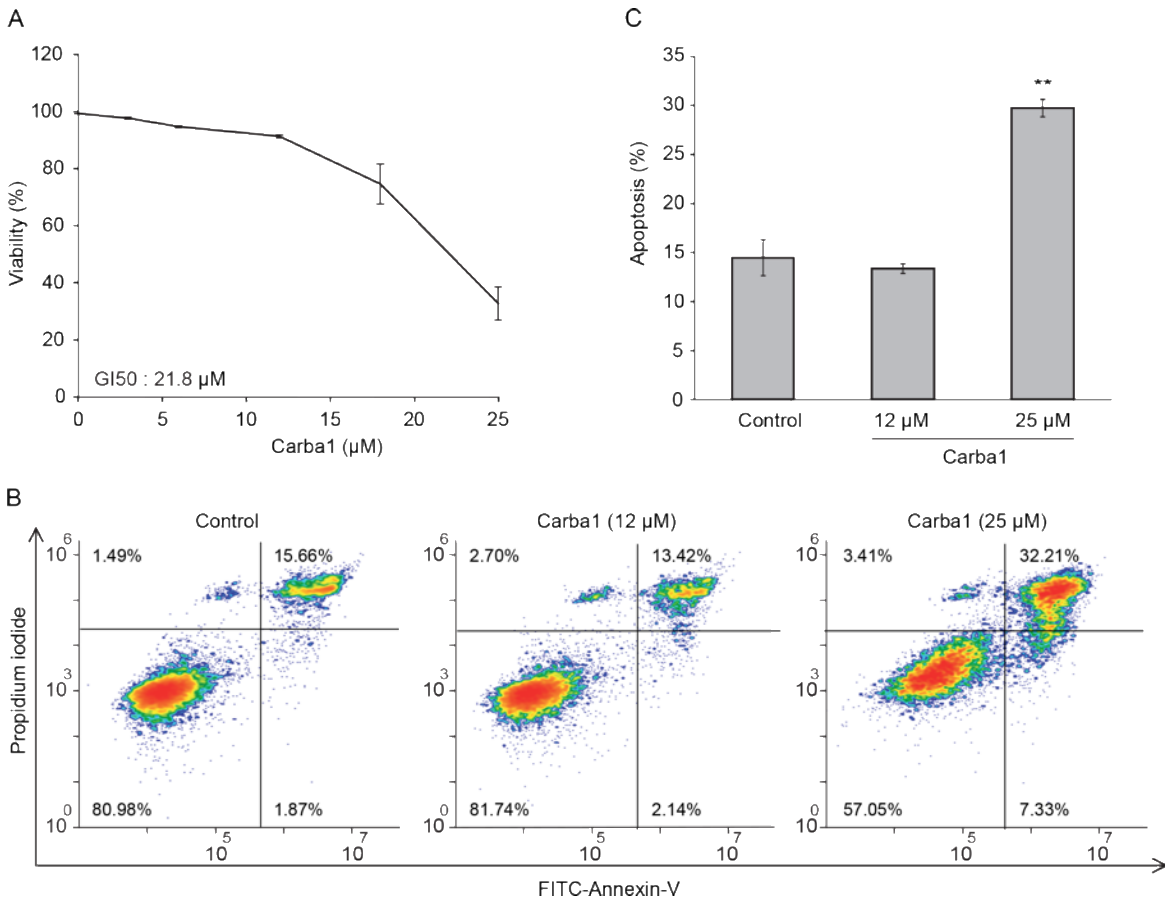
Carba1 has a low cytotoxicity when applied at high concentrations



NCI 60 screen : low toxicity

Analysis on a normal cell line (RPE-1) : low toxicity

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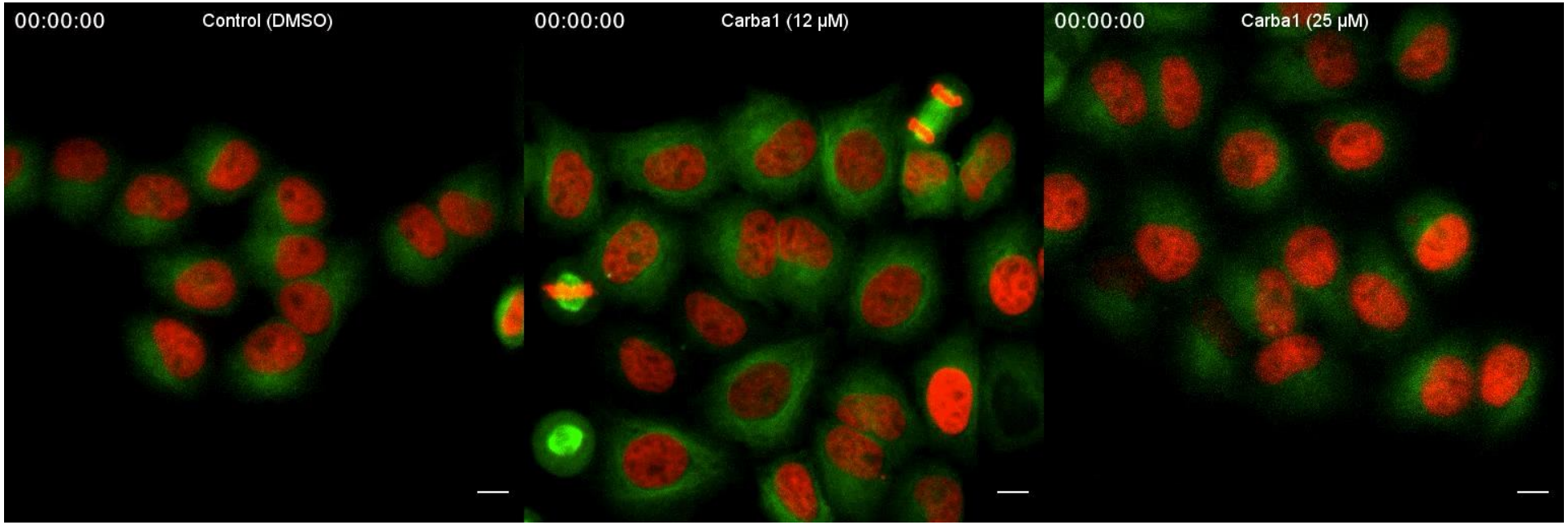
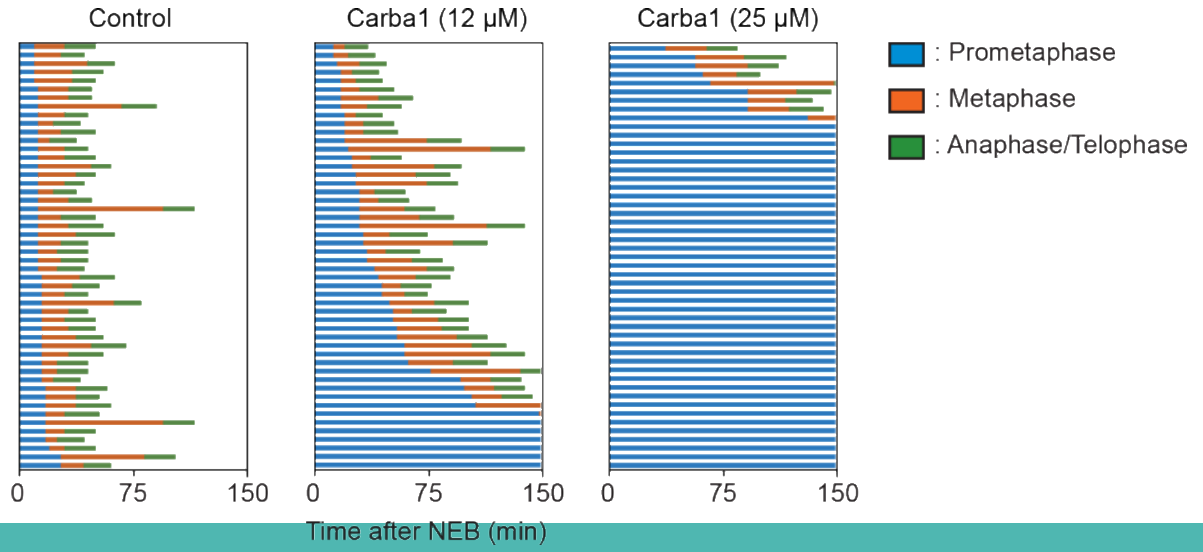
Cell-cycle progression is blocked at mitosis by Carba1





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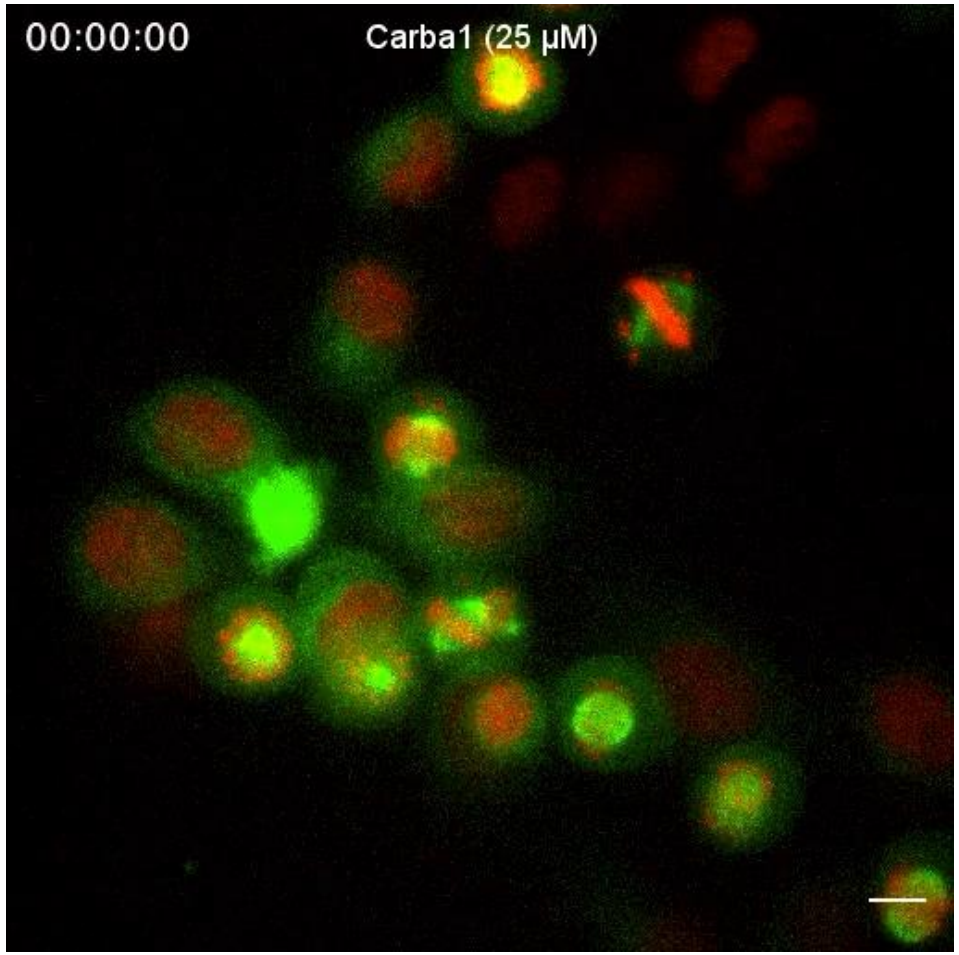
A high dose of Carba1 induces mitotic catastrophes



A cytotoxic dose of Carba1 induced a very long duration of mitotic arrest, followed by mitotic catastrophe

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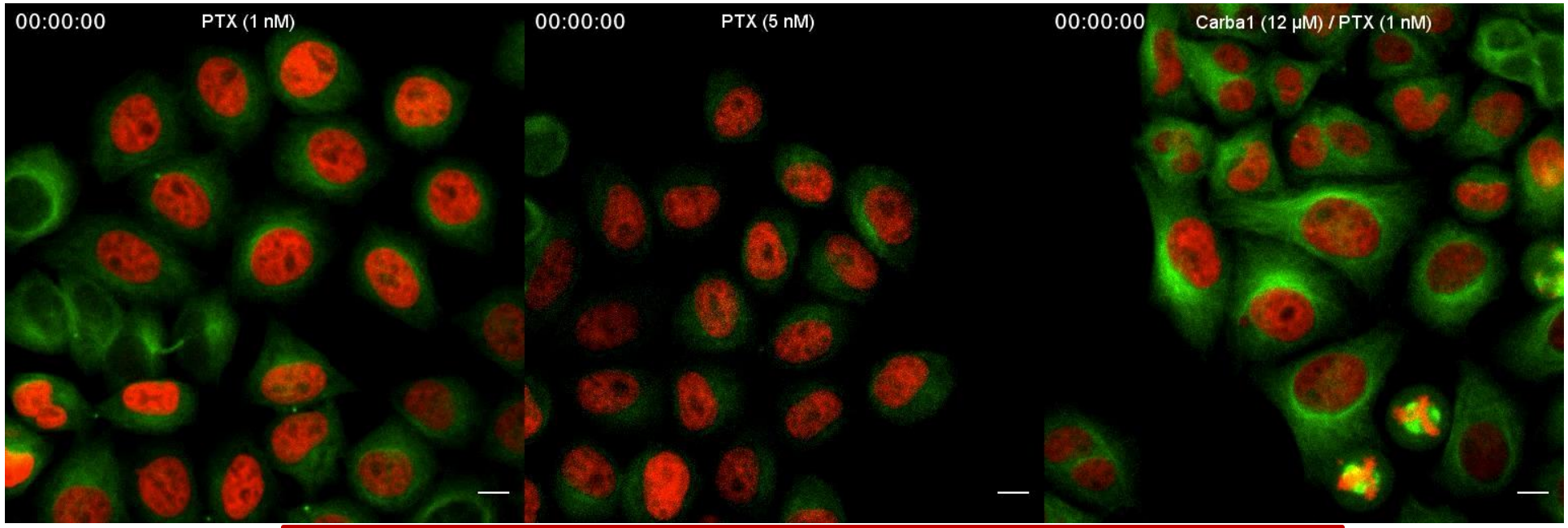
Carba1 Increases PTX Effects on Mitosis



PTX 1 nM	=	DMSO

Toxic doses

PTX 5 nM Aberrant mitosis then mitotic slippage	≠	Carba1 25 µM Prolonged metaphase arrest then mitotic catastrophe

PTX 1 nM + Carba1 12 µM Aberrant mitosis then mitotic slippage	=	PTX 5 nM Aberrant mitosis then mitotic slippage

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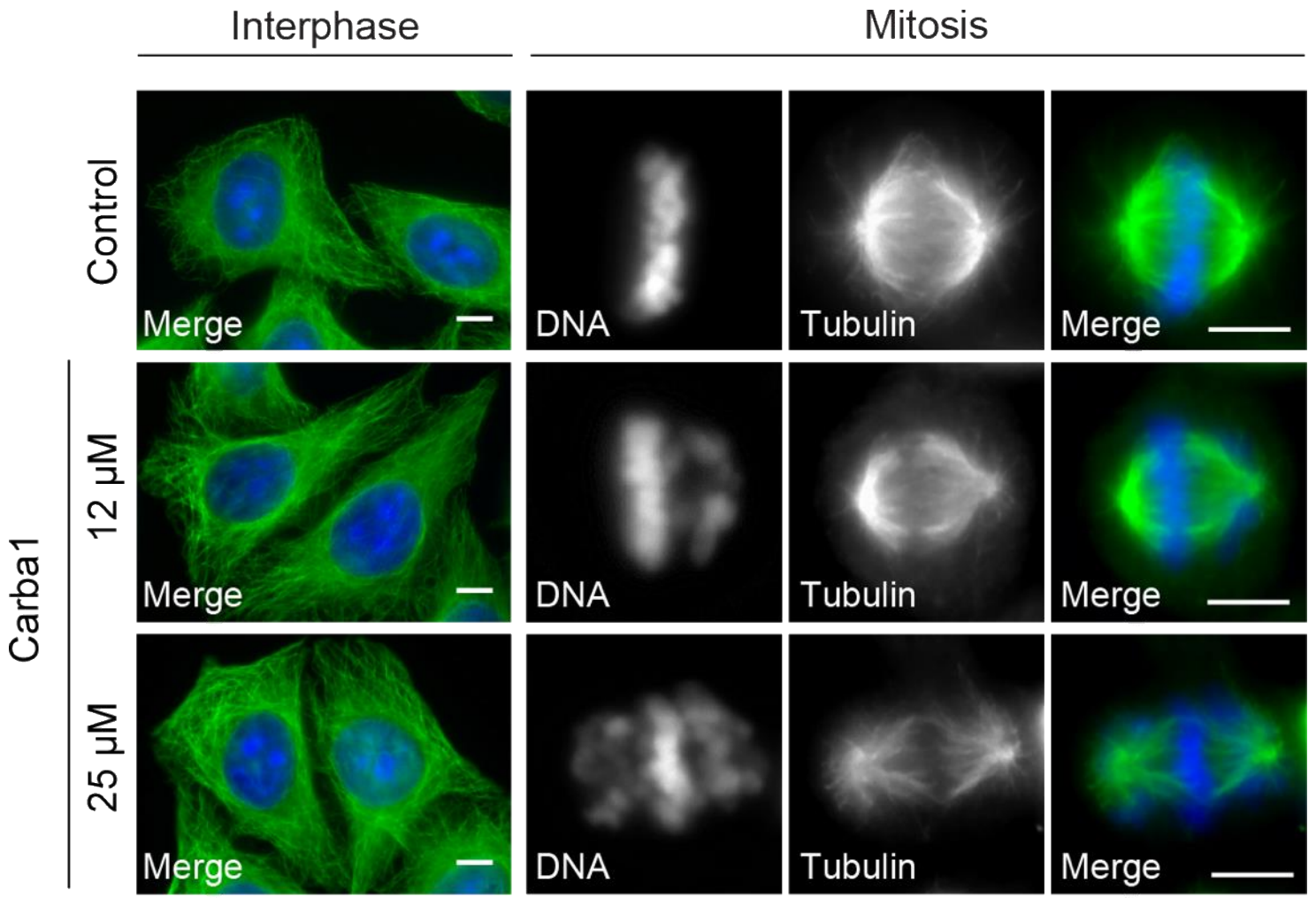
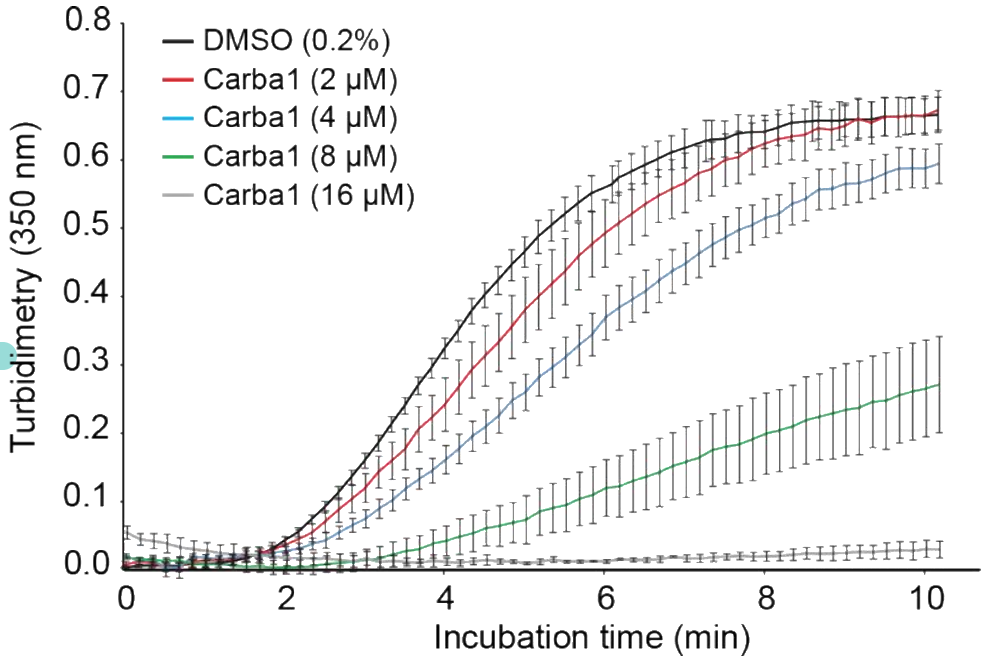
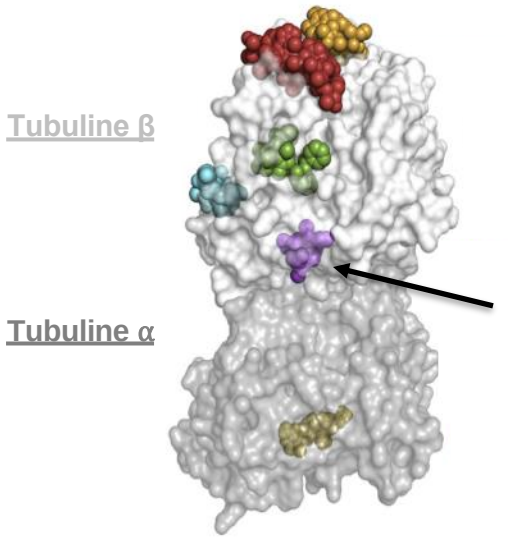
Carba1 target(s)?

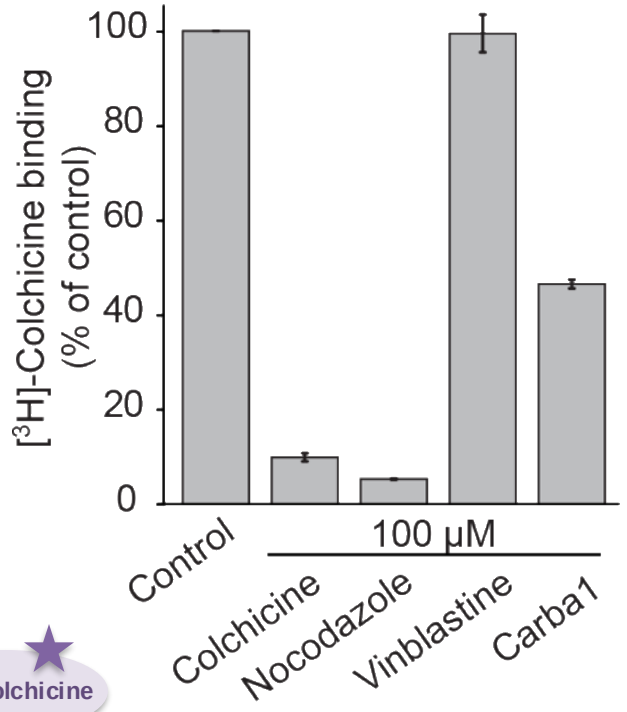


Tubulin?
Protein involved in mitosis regulation?

Carba1 inhibits in vitro pure tubulin polymerization by binding to the colchicine site of tubulin







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How a microtubule depolymerizing agent can synergize with a stabilizing agent?

- Carba1 binding on tubulin induces allosteric modifications that facilitate PTX binding?
- Carba1 binding on microtubule extremity facilitates PTX accumulation inside the microtubule

  - Collaboration with Fernando Diaz, Madrid
  - Collaboration with Anna Akhmanova, Utrecht

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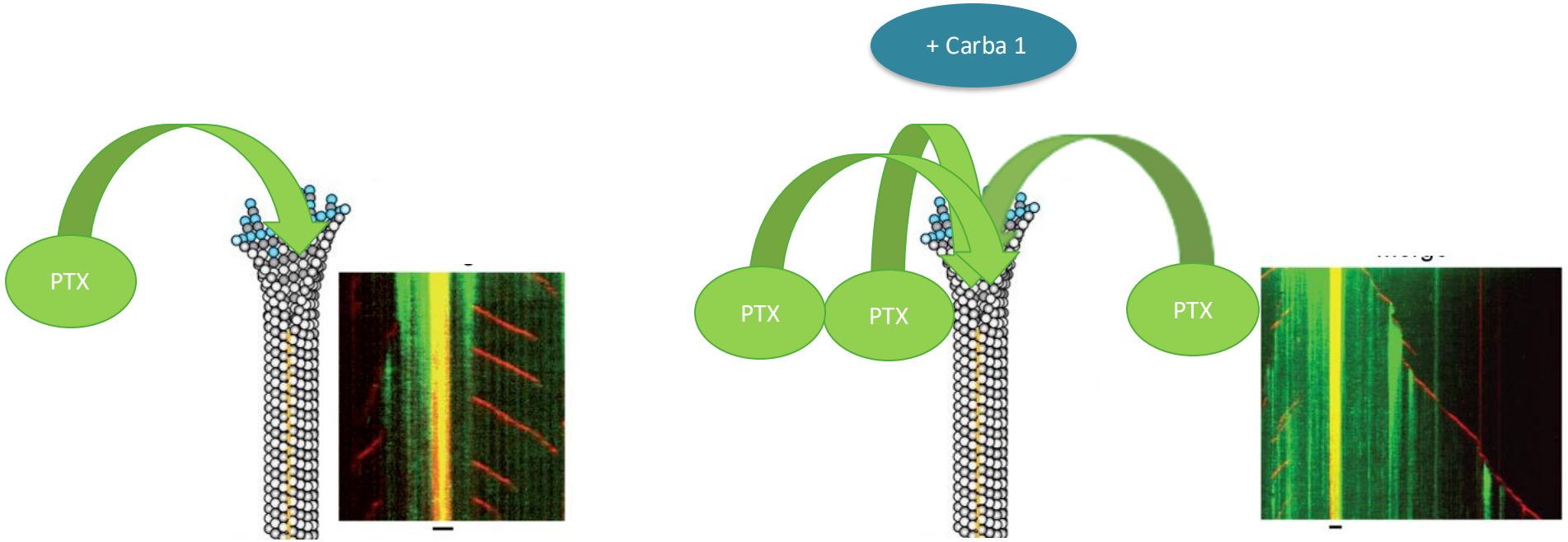
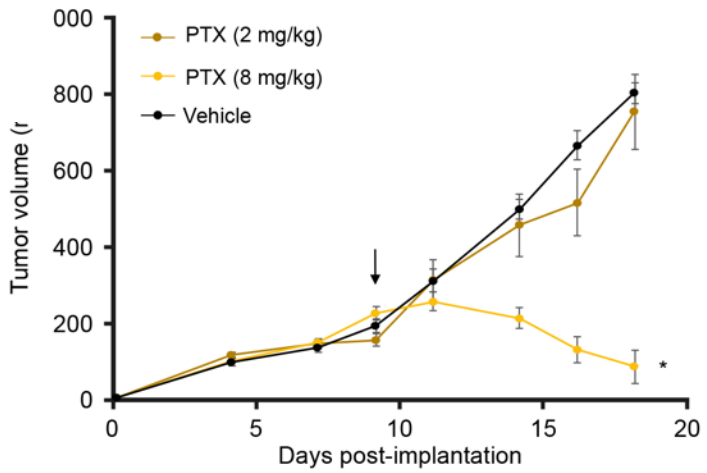
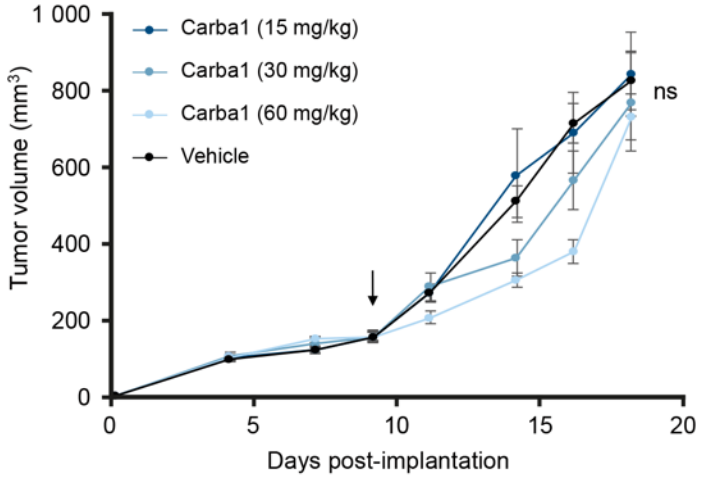
Microtubule growth perturbations trigger taxane accumulations

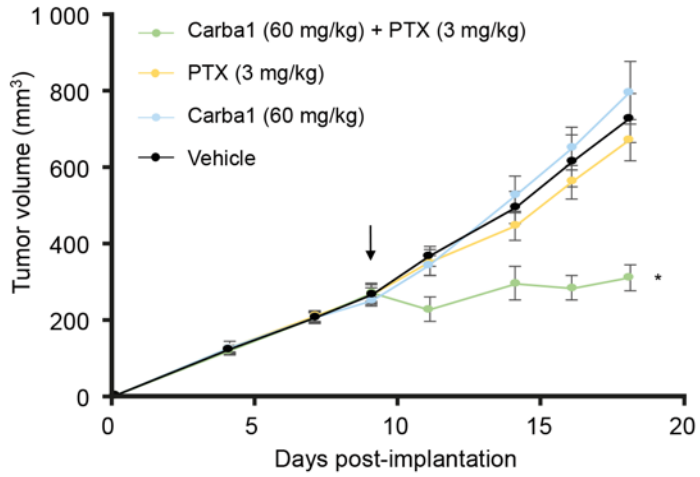


Carba1's synergistic mechanism involves subtle alterations in MT dynamics at the growing ends, leading to the formation of lattice regions that enhance the accumulation of compounds binding to the taxane site

Carba1 and PTX Act Synergistically to Reduce Tumor Growth In Vivo







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Conclusions #2

- Carba 1 is new PTX sensitizer, with low toxicity
- Our result pave the way for new therapeutic strategies combining low doses of microtubule targeting agents with opposite mechanisms of action
- Target ID after phenotypic screens is time-consuming
- Be perseverant…(target ID, animal models, publication, etc.)
- Do not forget to patent

Lauralie Peronne

cancers

MDPI

Article

Two Antagonistic Microtubule Targeting Drugs Act Synergistically to Kill Cancer Cells

Lauralie Peronne [1], Eric Denarier [2], Ankit Rai [3], Renaud Prudent [1], Audrey Vernet [1], Peggy Suzanne [4], Sacnicté Ramirez-Rios [1], Sophie Michallet [1], Mélanie Guidetti [5], Julien Vollaire [5], Daniel Lucena-Agell [6], Anne-Sophie Ribba [1], Véronique Josserand [5], Jean-Luc Coll [5], Patrick Dallemagne [4], J. Fernando Díaz [6], María Ángela Oliva [6], Karin Sadoul [1], Anna Akhmanova [3], Annie Andrieux [2] and Laurence Lafanechère [1,*]

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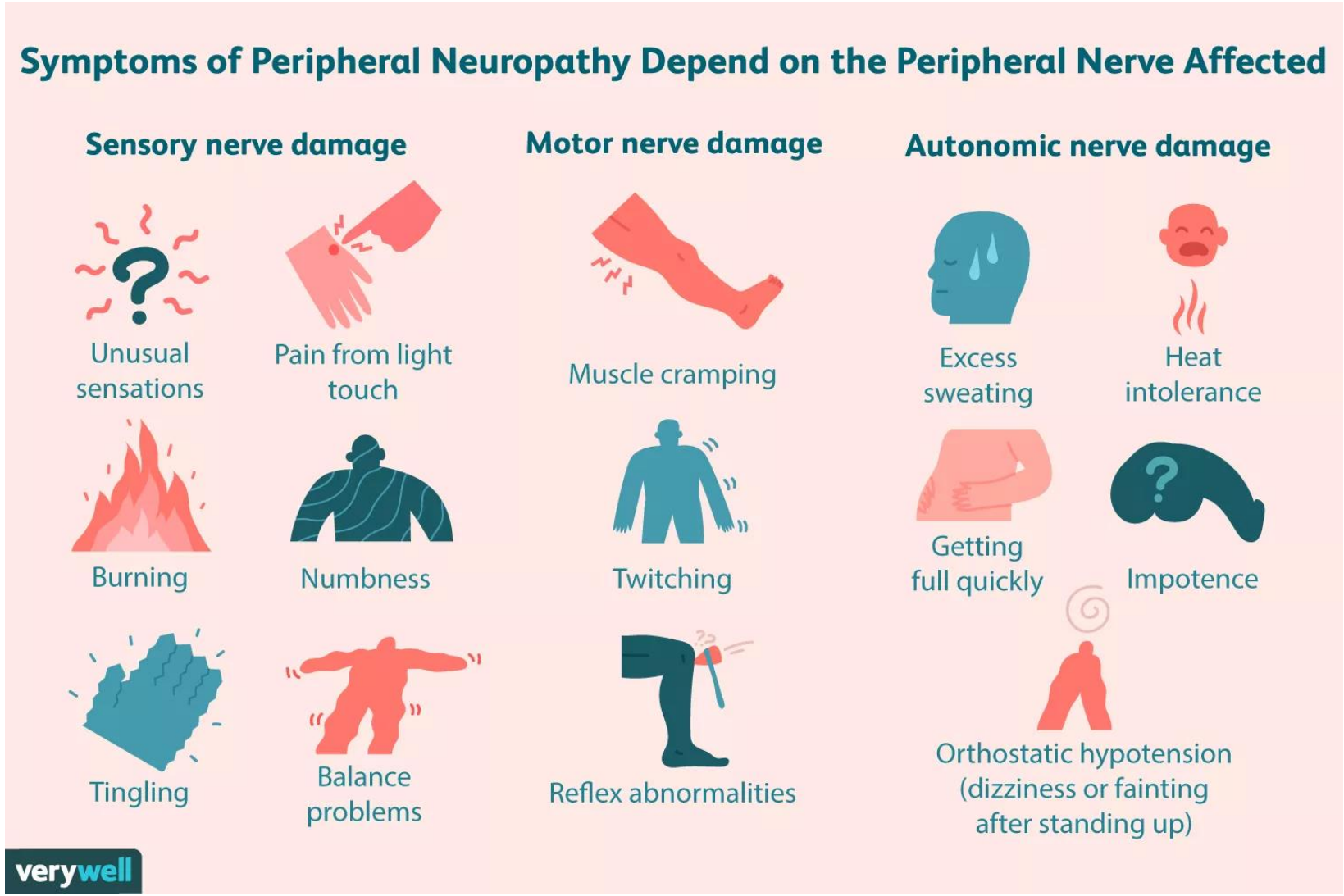
Does Carba1 mitigate the undesirable side effect, such as peripheral neuropathies, associated with high doses of Paclitaxel ?

Neuropathies: symptoms

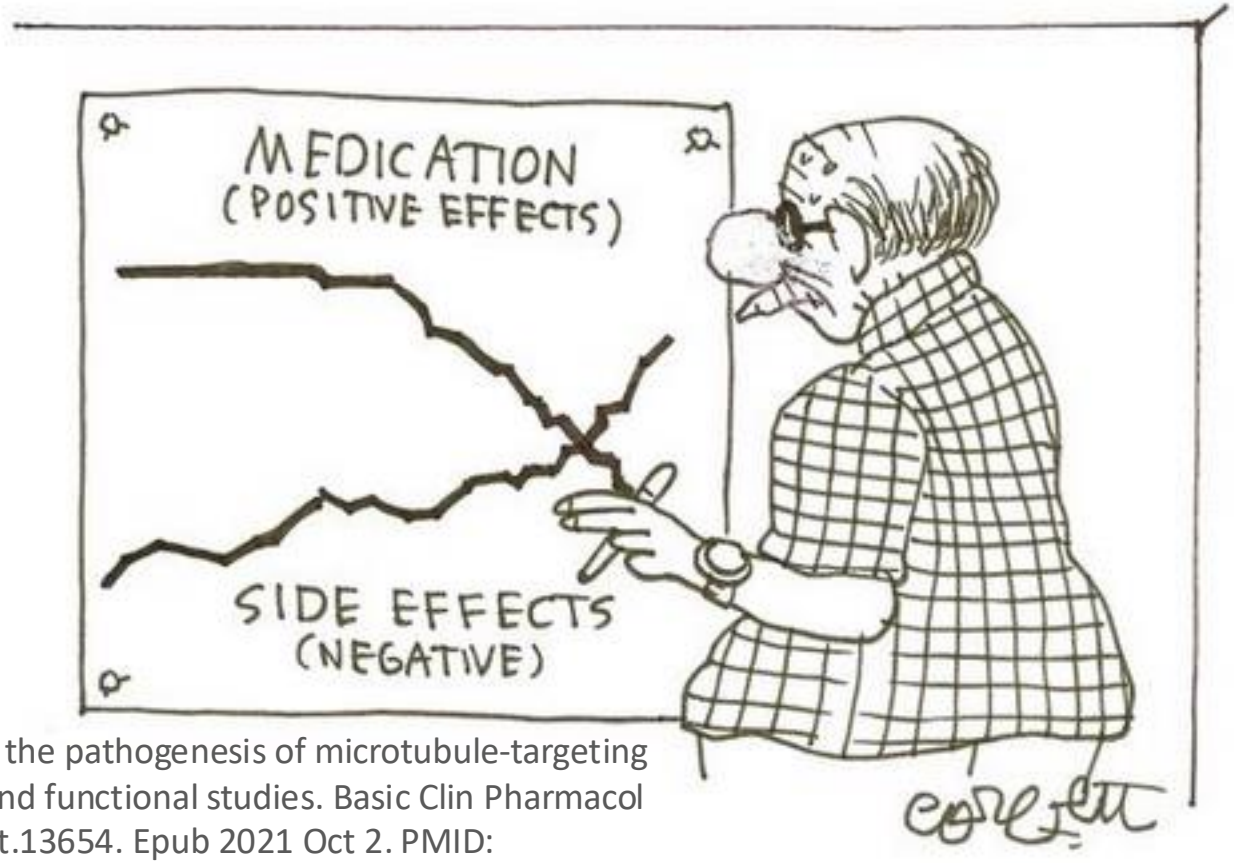


CIPN : a treatment-limiting adverse effect of anticancer therapy that complicates the lifestyle of many cancer survivors.

A frequent adverse effect : 80 % of the patients
Symptoms persist chronically in 30% of patients

models can more faithfully mimic target tissues and translate to patient experience.[94]

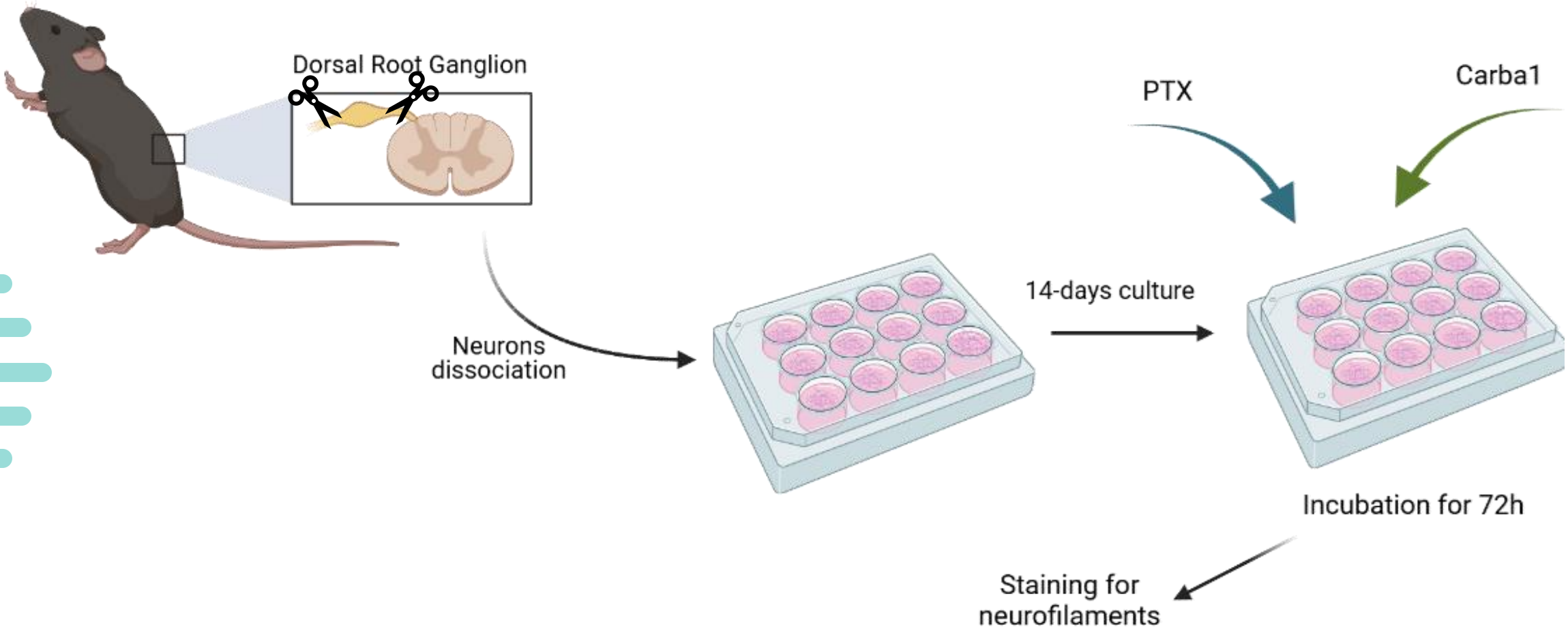
Considering that the decrease in cancer mortality is paralleled by an increasing number of cancer survivors who are prone to late effects of therapy, it is essential for the scientific community to develop standardized tools for the prediction, management, and treatment of patients genetically susceptible to CIPN. The application of pharmacogenetics to the study of CIPN will contribute to this goal.



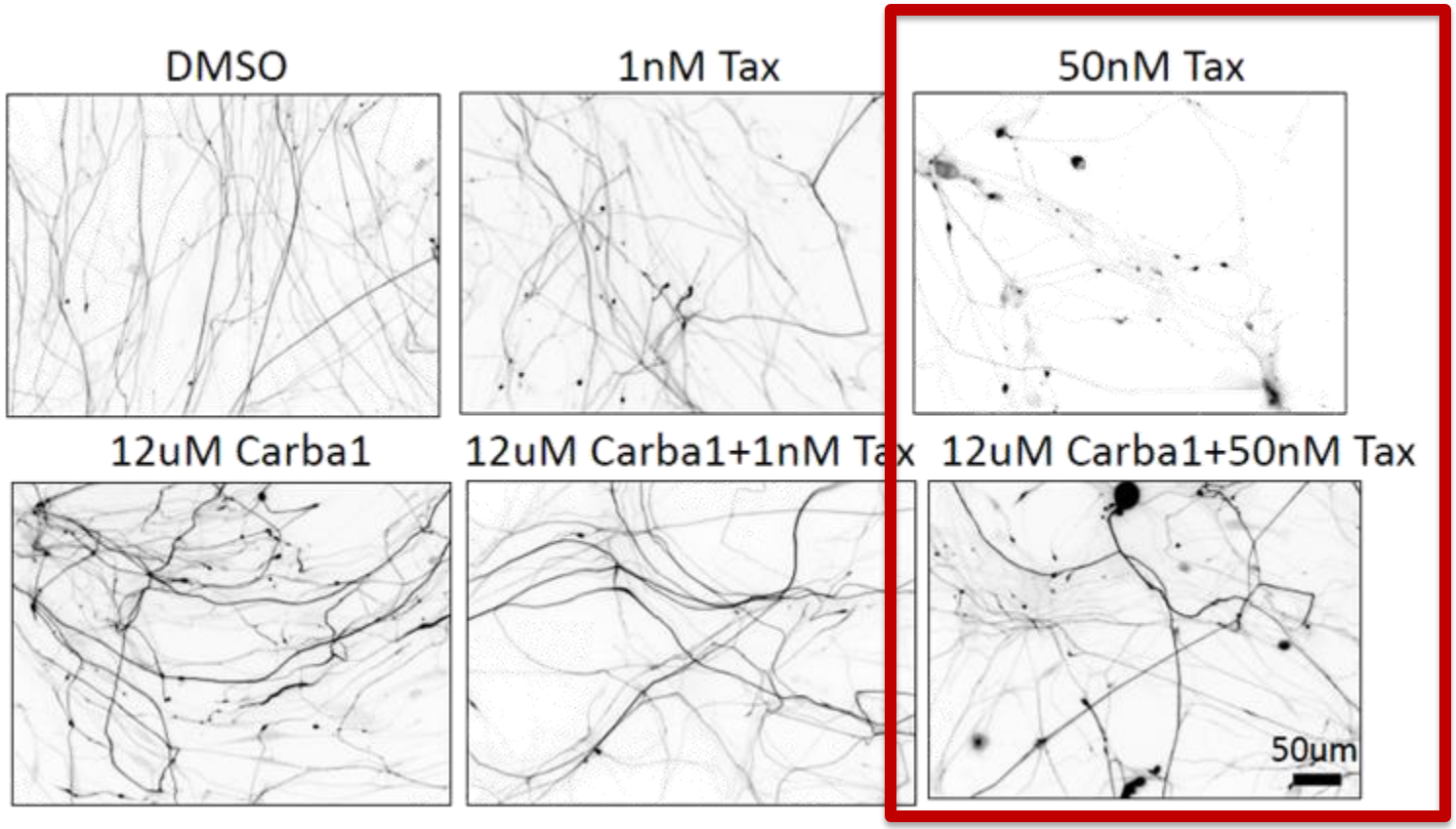
Chua KC, El-Haj N, Priotti J, Kroetz DL. Mechanistic insights into the pathogenesis of microtubule-targeting agent-induced peripheral neuropathy from pharmacogenetic and functional studies. Basic Clin Pharmacol Toxicol. 2022 Jan;130 Suppl 1(Suppl 1):60-74. doi: 10.1111/bcpt.13654. Epub 2021 Oct 2. PMID: 34481421; PMCID: PMC8716520.

Carba1 on DRG neurons

Coll. Francesca Bartolini & Maria Elena Pero, Columbia University



Carba1 protects neurons from PTX-induced degeneration



Less degeneration and neuronal fragmentation with Carba1

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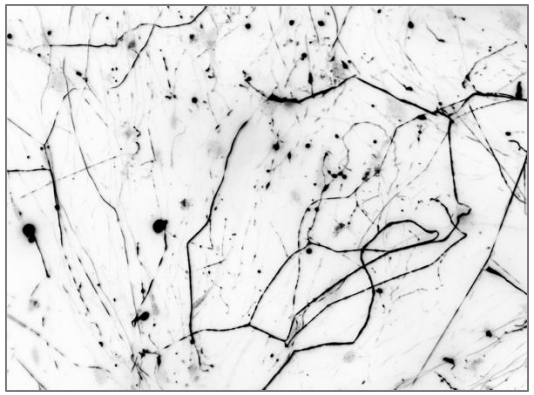
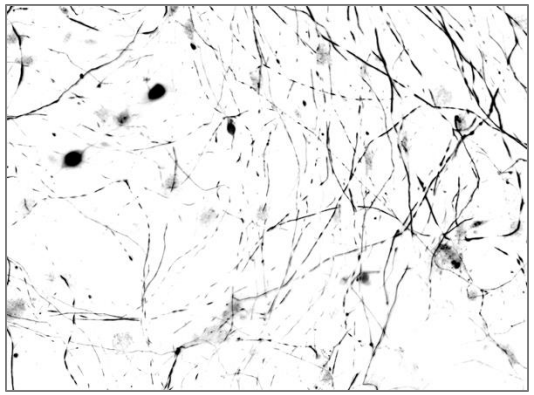
Carba1 protects neurons from Cisplatin-induced degeneration

DMSO

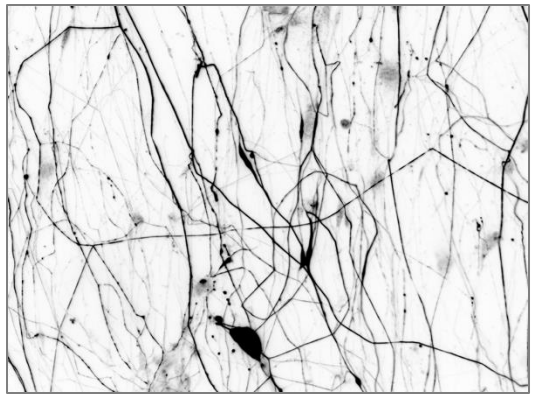
5 µM Cis



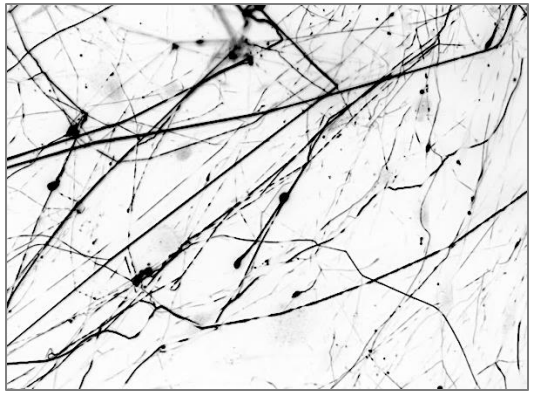
10 µM Cis



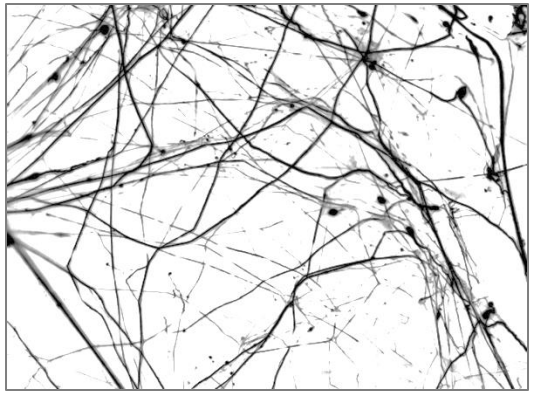
12 µM Carba1



5 µM Cis + 12 µM Carba1



10 µM Cis + 12 µM Carba1



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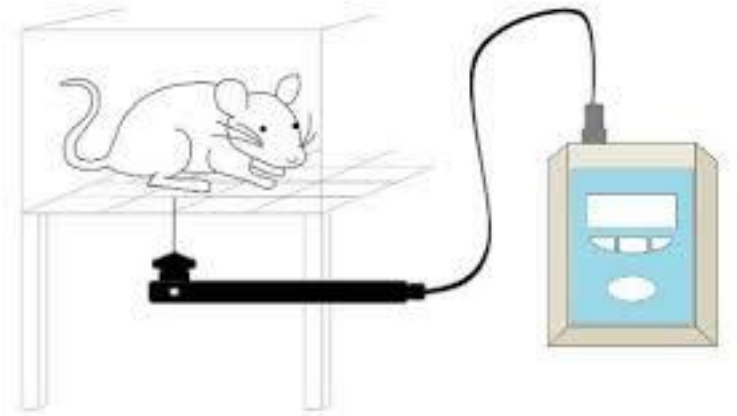
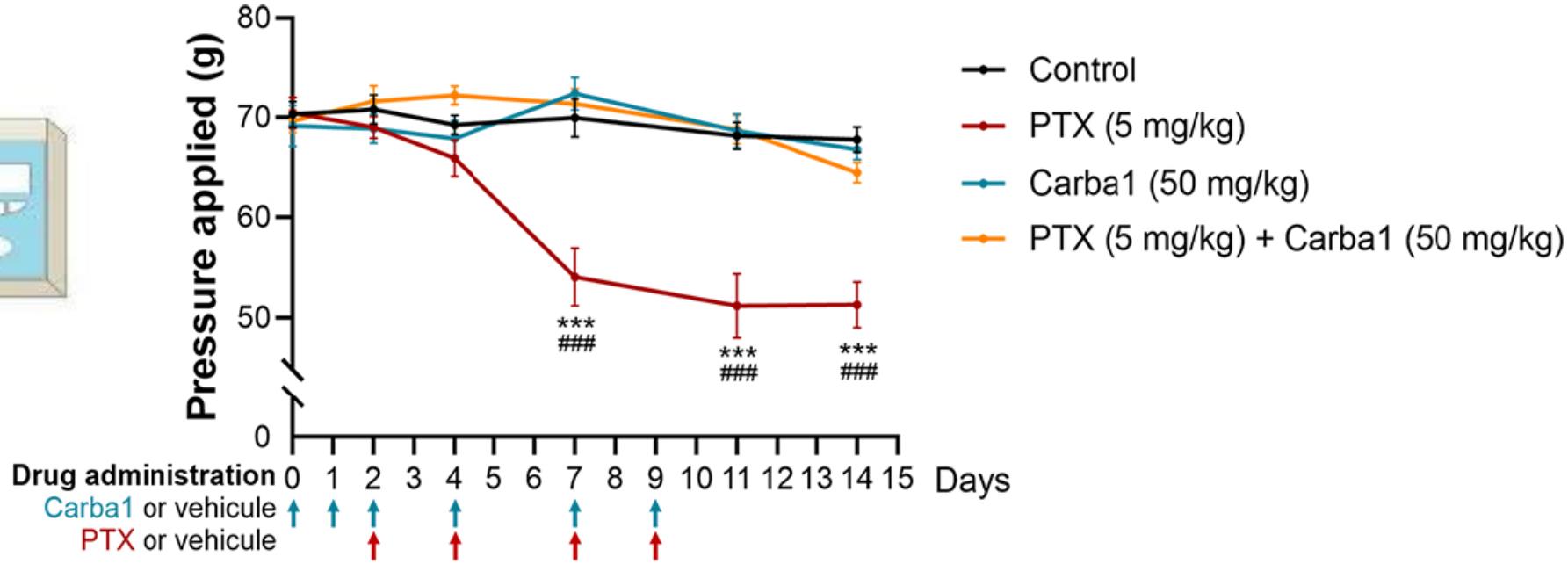
Carba1 prevents Paclitaxel-induced neuropathy in vivo





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Carba1 neuroprotection : MoA

Carba1 protects neurons from degeneration induced by :

- PTX, which targets tubulin directly
- Cisplatin, which targets DNA
- Bortezomib, which targets proteasome

➢ the neuroprotective activity results from activation of a target other than tubulin

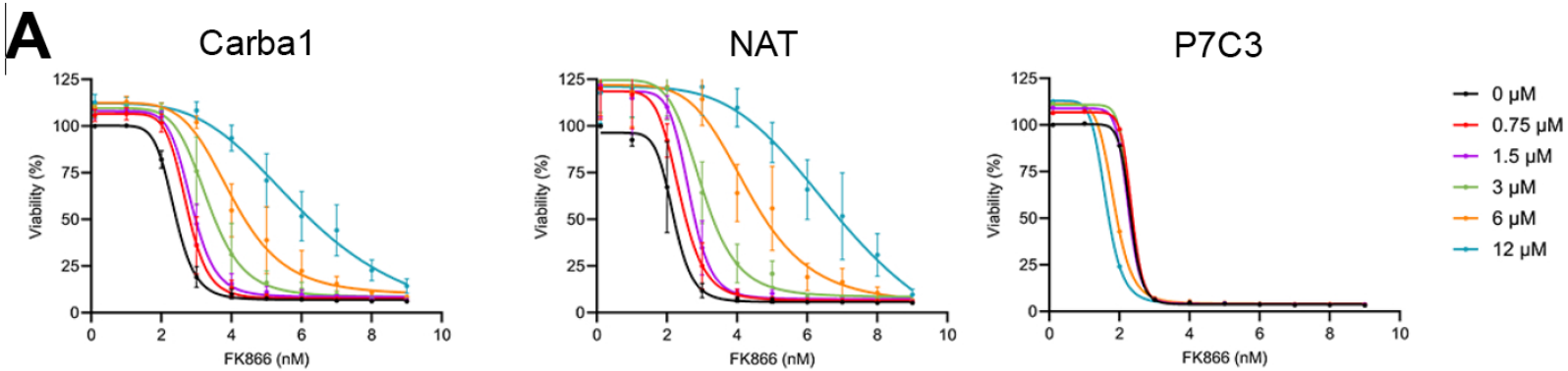
➢ the mechanism underlying this neuroprotection is generally beneficial to neuronal health

Neurons are the most energy-demanding cell type and bioenergetic failure is thought to be one of the main contributor of neuronal degeneration :

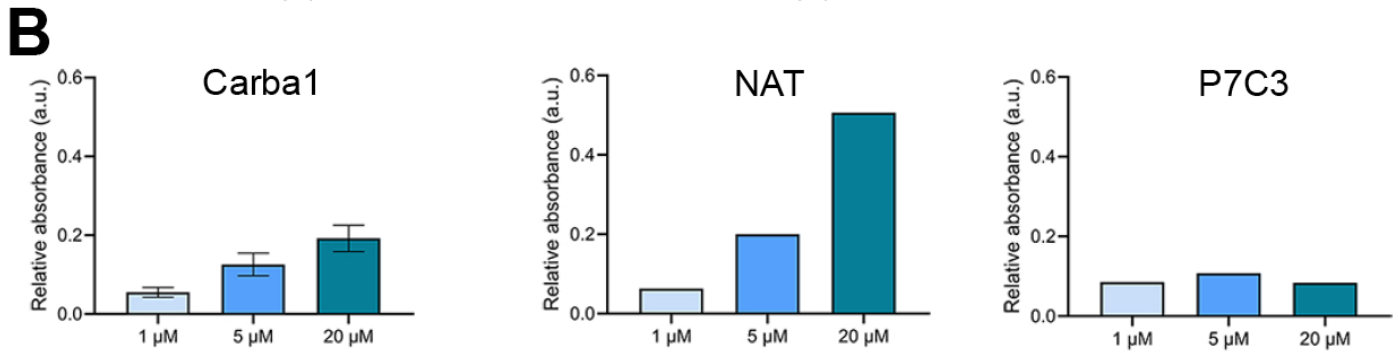
➢ we have demonstrated that Carba1 is an activator of NAMPT, an enzyme involved in the NAD salvage pathway

NAMPT is the target of Carba1

HeLa cells/ FK866

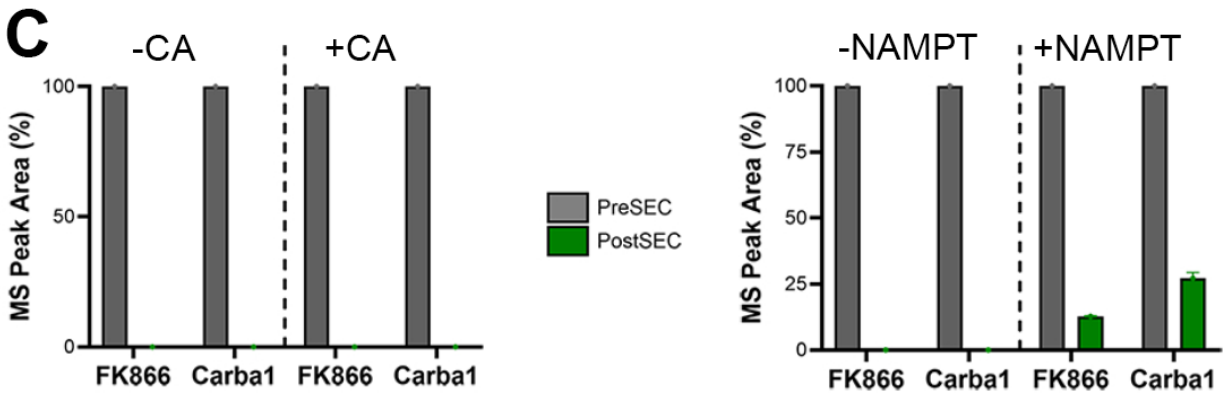


NAMPT activity in vitro



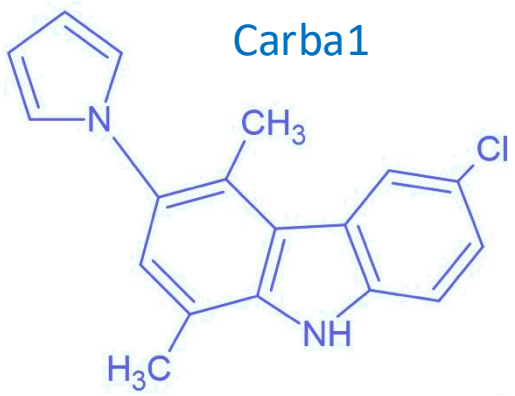
NAMPT is the target of Carba1, of NAT and not of P7C3

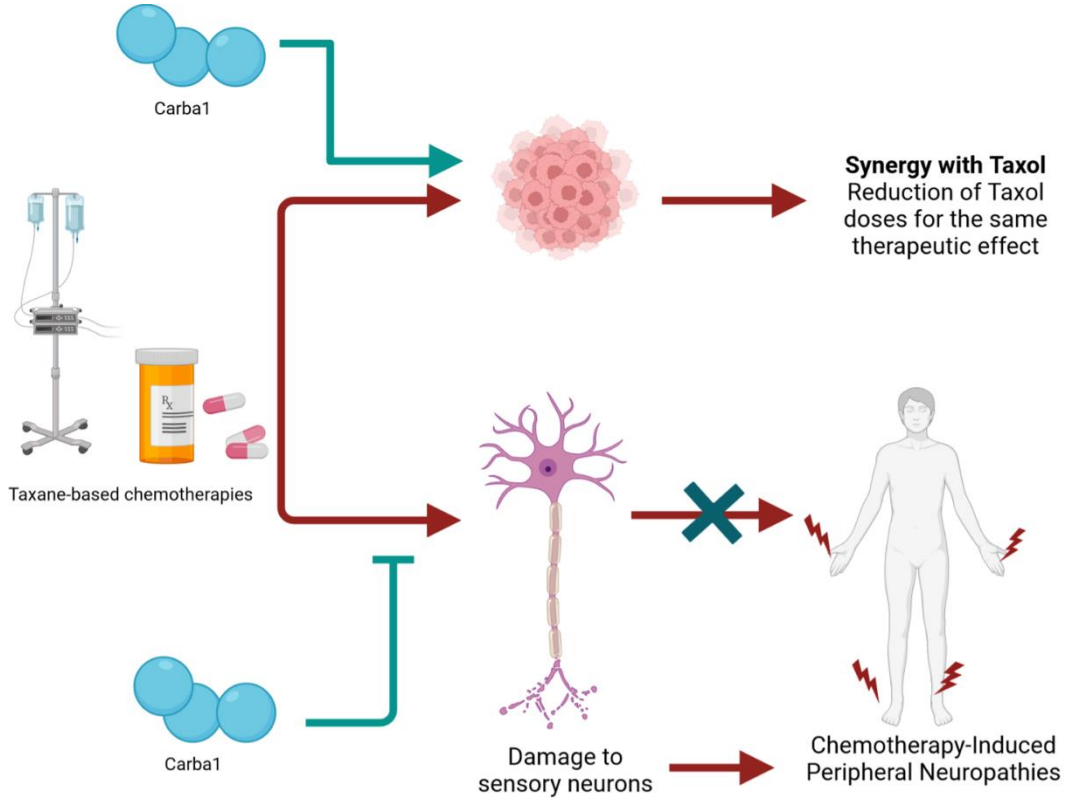
NAMPT binding



CA & NAMPT 3 µM
Carba1 & FK866 10 µM

Carba1 improves taxane therapy and prevents neuropathy





- A new patent
- A new publication
- A new start-up!

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Saxol Neuroprotecting Patient's Future

https://saxol.eu

Conclusions #3

- A drug often (always?) has several targets
- Understanding the mechanism of action always takes time ...
- Patent, Patent, Patent
- Solid support for developing an innovation through the prematuration and maturation programs

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