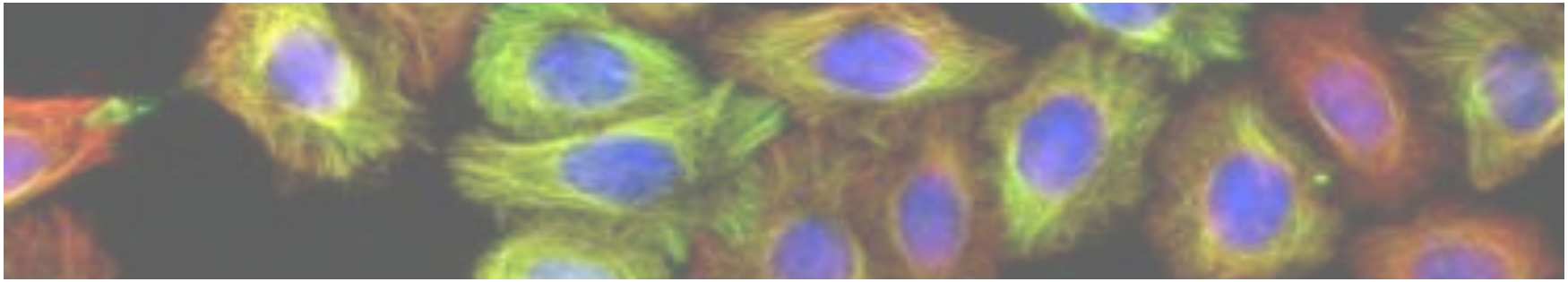
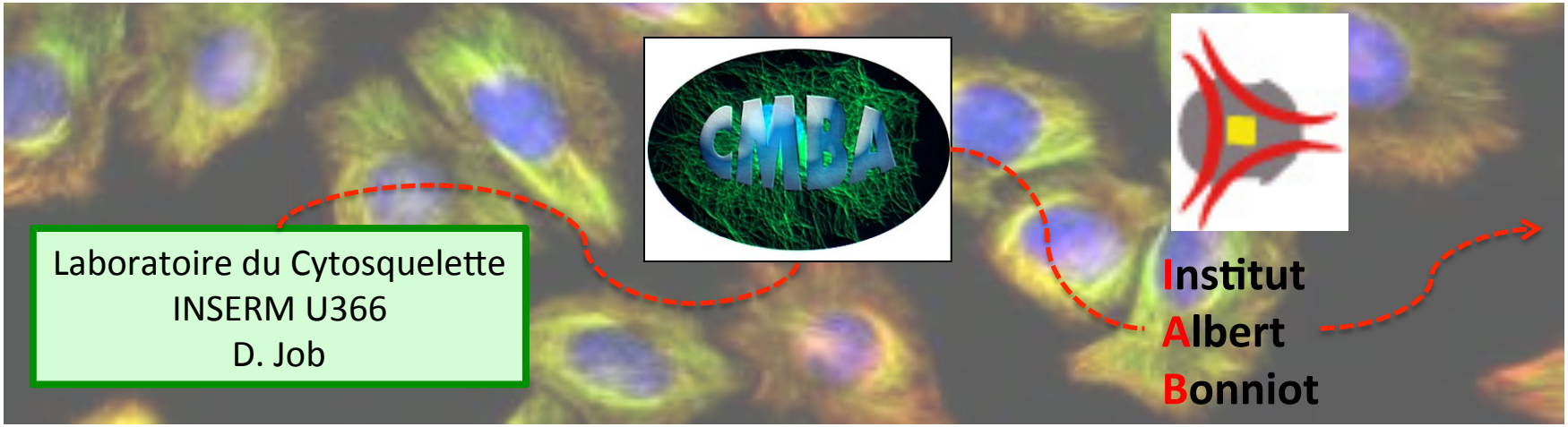


Itinéraire d'une petite molécule aux propriétés anticancéreuses prometteuses

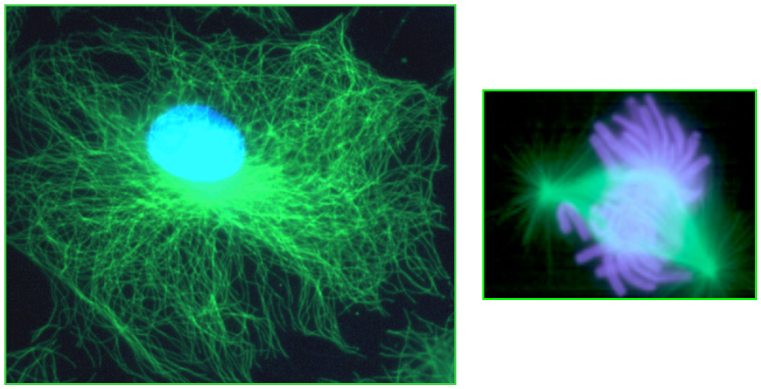
Laurence Lafanechère

Institut Albert Bonniot
Equipe Polarité, Développement et Cancer Grenoble, France (Direction M. Billaud)

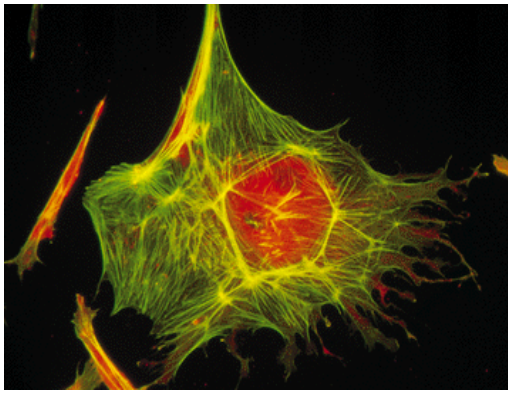


The cytoskeleton : a target for cancer therapy

Loss of proliferation control



Gain of motility (metastasis)



Microtubules

Actin microfilaments

- Microtubules and actin microfilaments are dynamic networks
- The cell closely controls and coordinates these networks dynamics

Mechanisms of regulation?

Post-traductionnal modifications
- of proteins that constitute the cytoskeleton, for instance tubulin
- of associated proteins, for instance cofilin (phosphorylation)

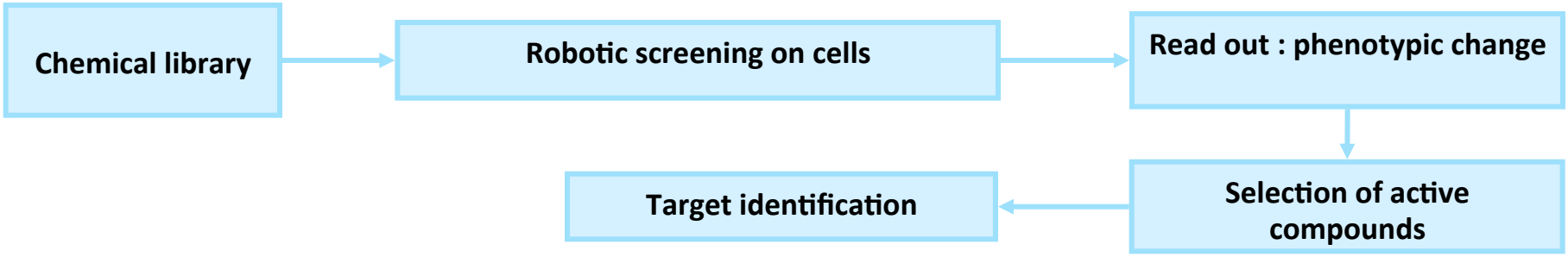
Research aims

- What is the role of cytoskeleton regulators in tumorigenesis?
- Can we find pharmacological agents targeting these regulators?

Small molecules targeting the cytoskeleton: selective tools for unraveling its functions

- Small molecules have been used successfully to probe biological mechanisms for many years (nocodazole).
- Valuable probes to study dynamic biological processes. Generally acting within minutes or even seconds, they can provide a high degree of temporal control over protein function.
- As they are also often reversible, they allow both rapid inhibition and activation.
- They can allow dose-dependent control of biological functions.

Chemical biology approaches allow to simultaneously find new leads and new effectors



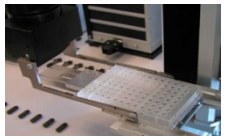
Small Molecule Inhibitor of Mitotic Spindle Bipolarity Identified in a Phenotype-Based Screen

Thomas U. Mayer,[1]* Tarun M. Kapoor,[1] Stephen J. Haggarty,[2,3] Randall W. King,[2] Stuart L. Schreiber,[2,3] Timothy J. Mitchison[1,2]

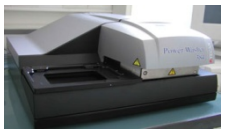
SCIENCE VOL 286 29 OCTOBER 1999

Search for new microtubule regulators using a cell based assay screen

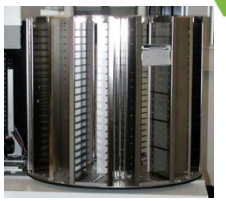
CMBA: a platform dedicated to cell-based assays



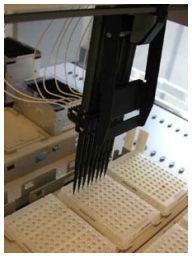
Robotic holder



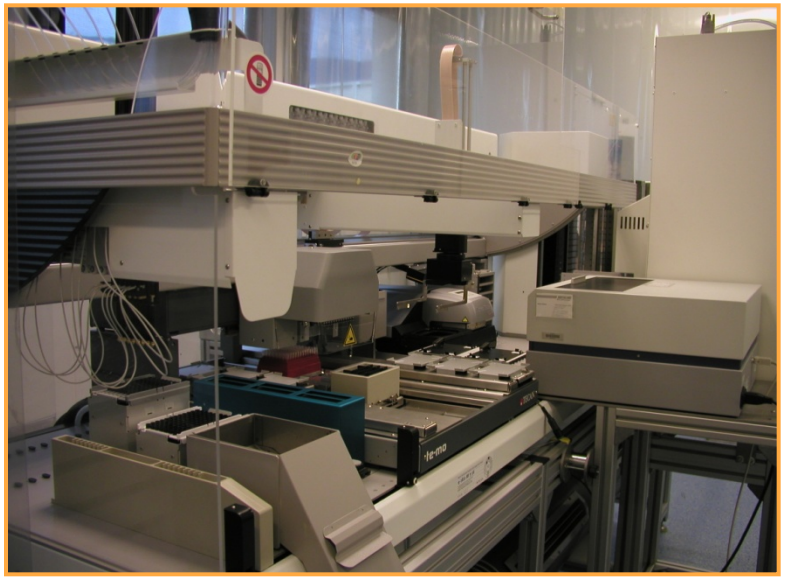
96 channel microplate washer



Storage tower



8 channel multipipettor

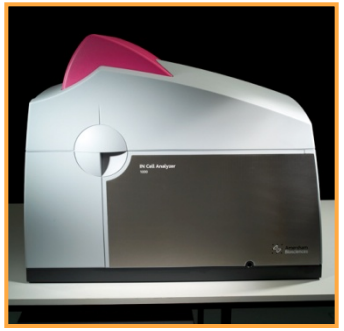


96 channel multipipettors

Multimode microplate reader

+ a chemical library of more than 30,000 diverse compounds

Cell incubator

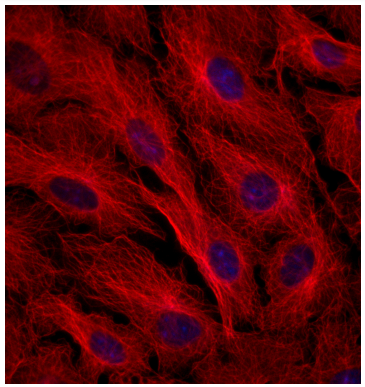


High content analysis imager

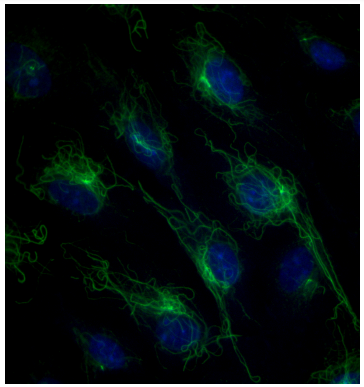
Emmanuelle Soleilhac

Caroline Barette

Search for new microtubule regulators using a phenotypic screen



Tyr Microtubules



Detyr Microtubules

A cell-based assay to select:

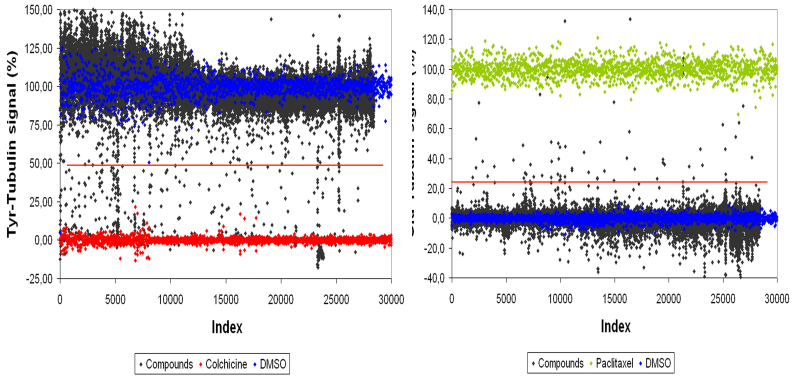
- microtubules stabilizing agents
- microtubules depolymerizing agents

Emilie Vassal

Catherine Pillet

Vassal et al., J. Biomol. Screen., 2006

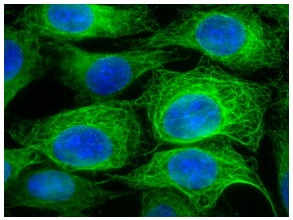
Screening of 30 880 molécules

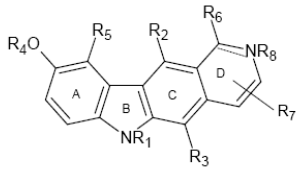


85 « dépolymerizing » compounds

20 « stabilizing » compounds

A stabilizing compound wih novel properties





Pyridocarbazole, Pyr1

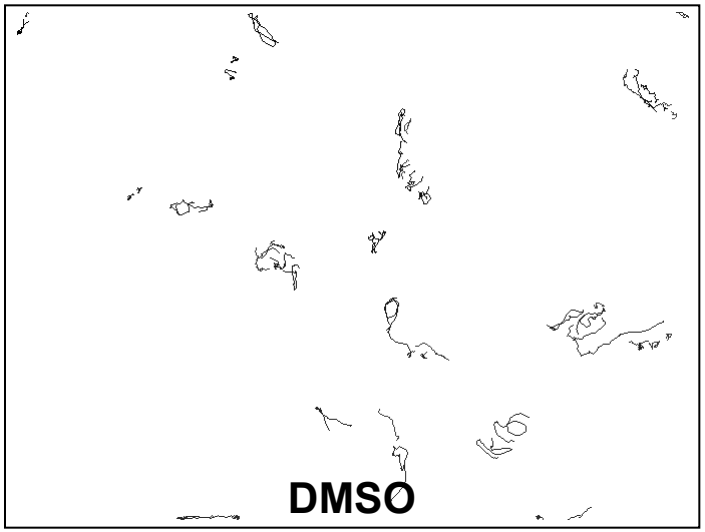
- Stabilizes **microtubules and** affects **actin microfilaments**
- **Without acting directly** on tubulin or actin
- Impedes cell motility
- Potential **anticancer and antimetastatic agent**

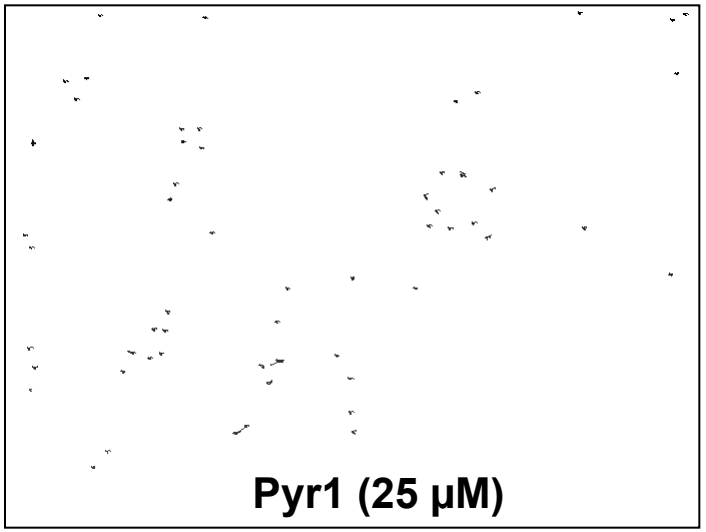
Patent WO201095042A2, national phases

Microtubule stabilization
Videomicroscopy analysis

Anne Martinez

Cell Motility
Videomicroscopy analysis

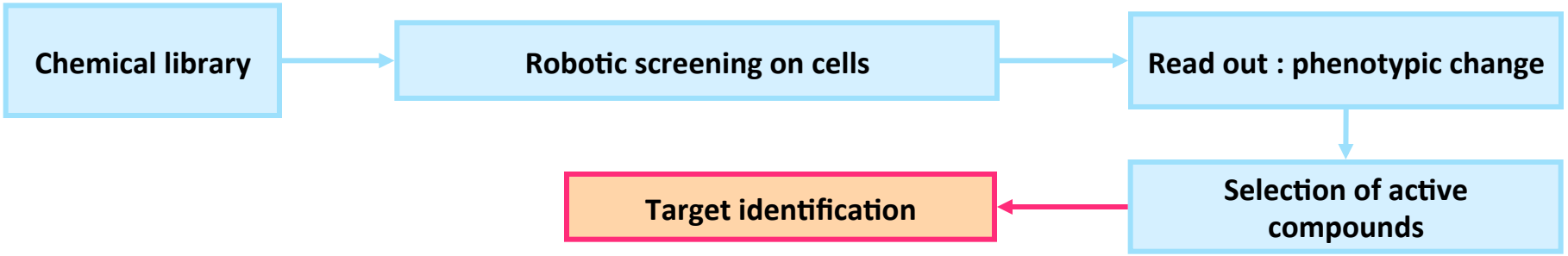




Pyr1 bloque la motilité des cellules

Coll. A. Beghin, O. Filhol-Cochet

Pyridocarbazole does not act directly on tubulin or actin : what is its target?



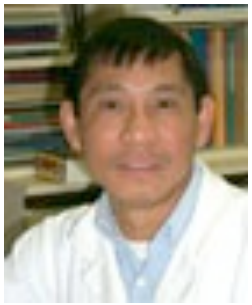
We were like Prince Charming, in Cinderella, …

… with only a tiny glass slipper,
"Pyr1", to find our a beautiful phenotype.

Hoping that our compound is not like these ugly slippers, which fit all feet, including the ugly step sisters' feet!

Isolation and identification of the target(s), using ligand based methods

- Structure/ ow where it is possible
- Affinity co extracts / identificat



Chi-Hung Nguyen

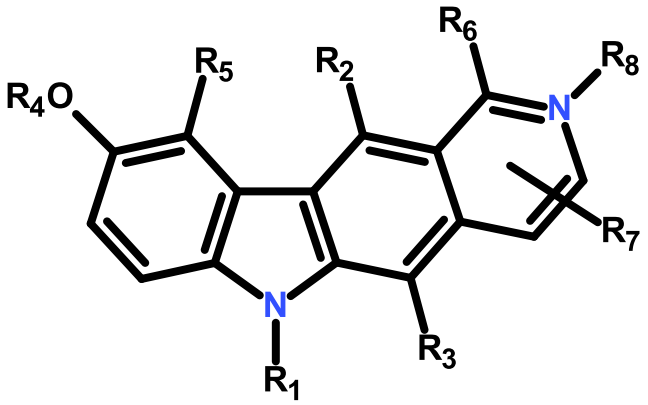
Coll. C-H Nguyen (Institut Curie), plateforme de protéomique (CEA)

Guess and test the putative target

Guess : the target is a kinase

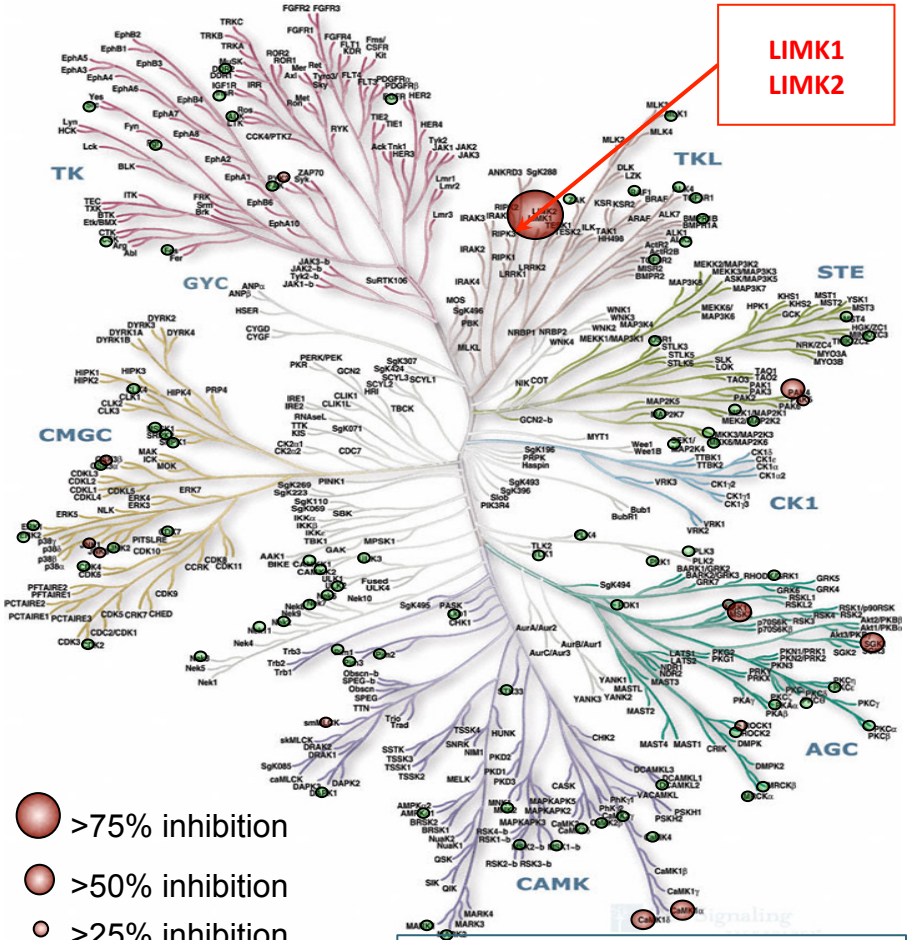
Renaud Prudent

Pyr1 : a New Highly Specific Inhibitor of LIM Kinase



Pyr1 = LIMINIB

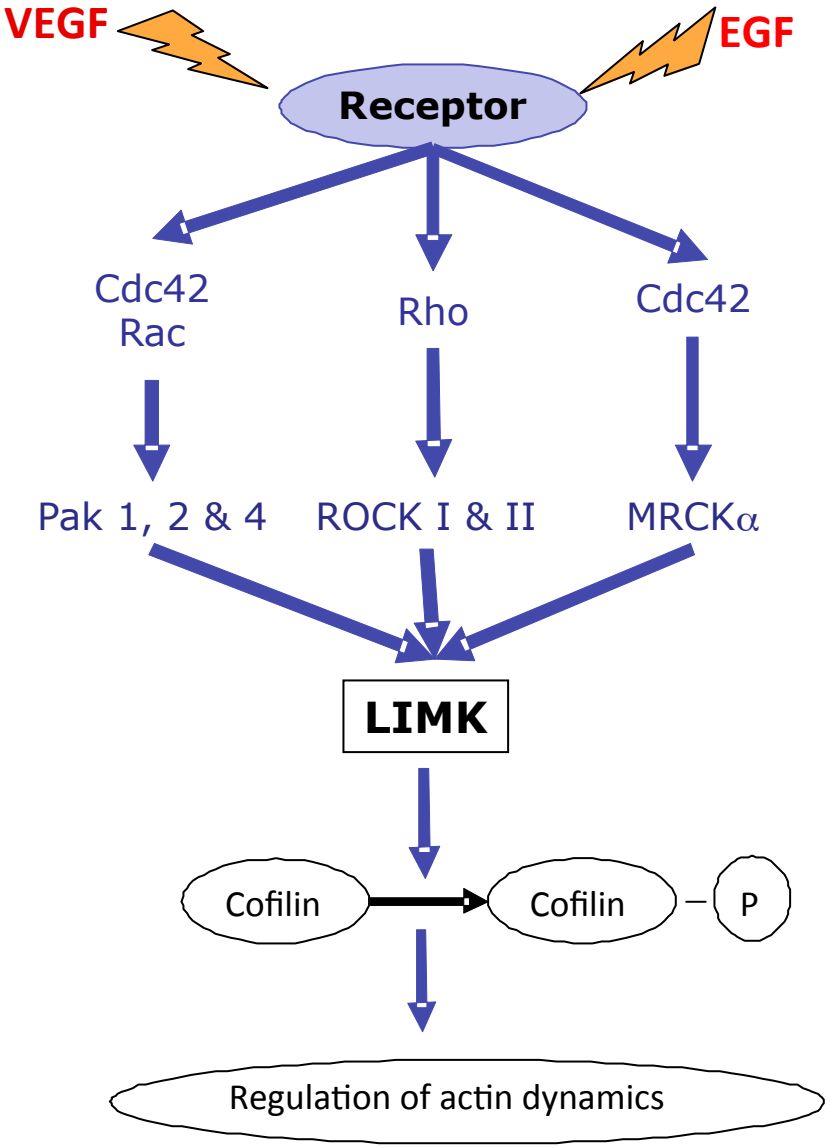
Renaud Prudent

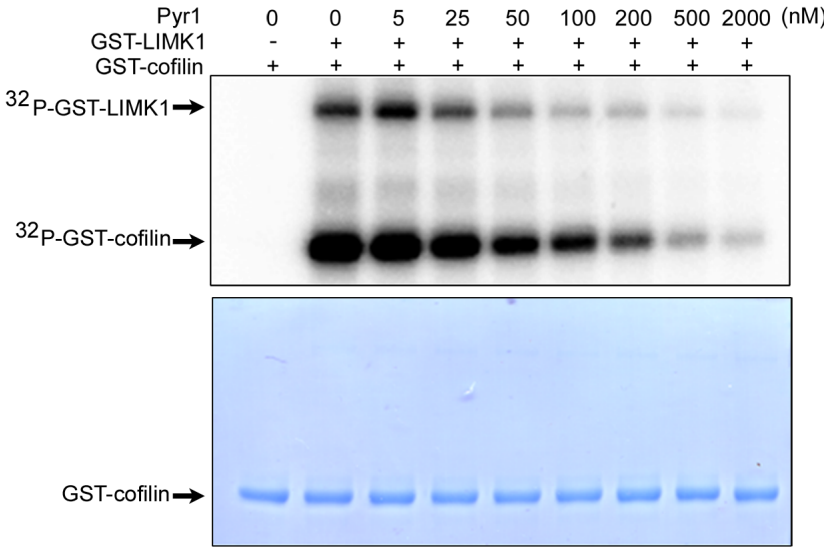


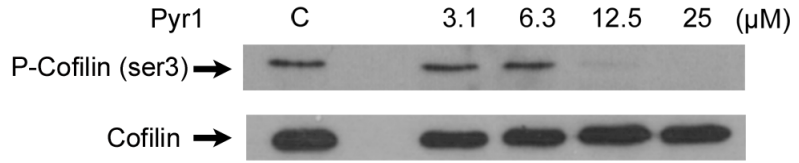
Inhibition of only one kinase out of 110 kinases tested

Coll. Stefan Knapp, Oxford

LIM Kinases : the last step in the cascade regulating actin dynamics through cofilin phosphorylation





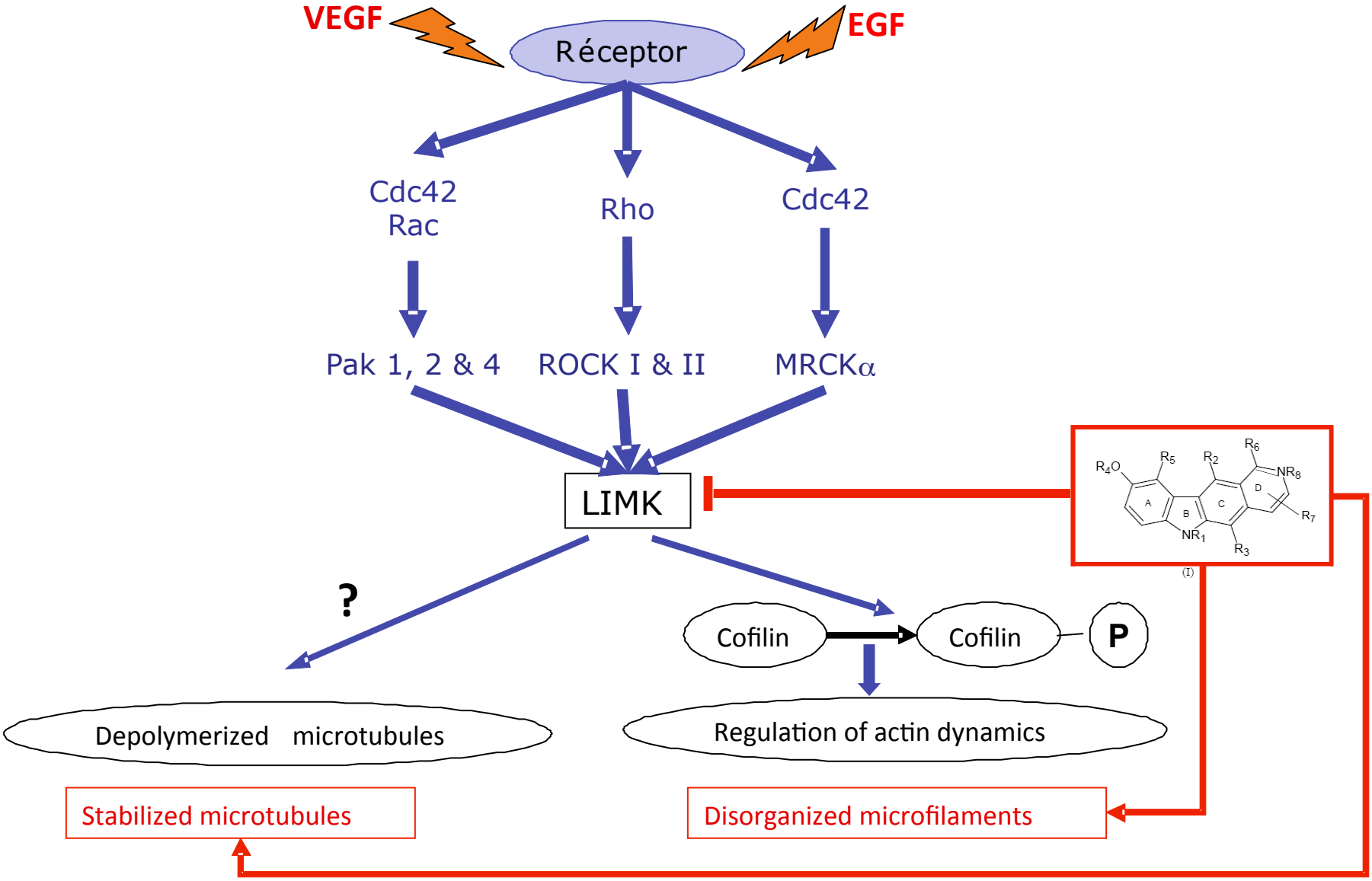


Pyr1 is an ATP competitive inhibitor (Ki = 60 nM) of LIMK1 and LIMK2, active both *in vitro* and *in cellulo*

LIMK inhibition causes microtubule stabilization

- The overexpression of LIMK1 can counteract the microtubule stabilizing effect of Pyr1
- A structurally different LIM Kinase inhibitor also stabilizes microtubules
- LIMK invalidation (shRNA) leads to microtubule stabilization

LIMK : a signaling node that controls both actin and microtubules dynamics



LIM Kinase: an Emerging Target for Cancer Chemotherapy

An increasing number of publications confirm that LIM Kinase is a target for cancer therapy

The cofilin pathway in breast cancer invasion and metastasis

NATURE REVIEWS | CANCER
VOLUME 7 | JUNE 2007 | 429

Weigang Wang, Robert Eddy‡ and John Condeelis‡§*

Abstract | Recent evidence indicates that metastatic capacity is an inherent feature of breast tumours and not a rare, late acquired event. This has led to new models of metastasis. The interpretation of expression-profiling data in the context of these new models has identified the cofilin pathway as a major determinant of metastasis. Recent studies indicate that the overall activity of the cofilin pathway, and not that of any single gene within the pathway, determines the invasive and metastatic phenotype of tumour cells. These results predict that inhibitors directed at the output of the cofilin pathway will have therapeutic benefit in combating metastasis.

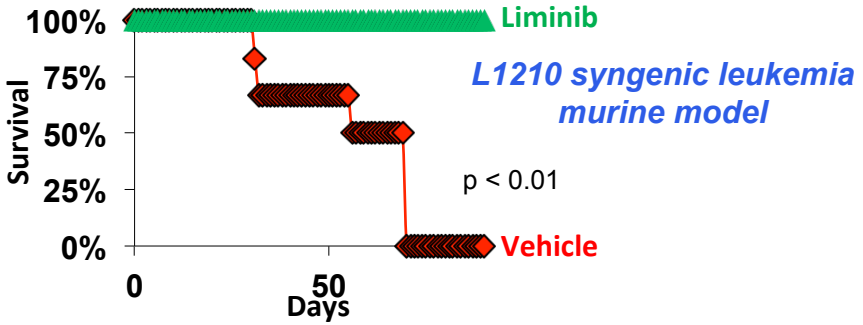
Current Cancer Drug Targets, 2012, 12, 543-560 543

Recent Findings Confirm LIM Domain Kinases as Emerging Target Candidates for Cancer Therapy

F. Manetti*

Pharmacological Development Status

✓ Therapeutic efficacy : *in vivo* proof of concept



Chloé Prunier

Coll. Équipes C. Dumontet et JL Coll

✓ Liminib is well tolerated in a 7 days murine toxicity study + large therapeutic window

✓ Pilot pharmacokinetics study realized

➢ Liminib exerts a potent antitumoral effect and displays favorable toxicity profile

Prudent R. *et al*, Cancer Research, septembre 2012

Further directions

- Investigate the mechanisms underlying LIMK inhibition and microtubule stabilization

- Use the compound to dynamically study the role of LIMK in cell migration and during development (Williams-Beuren)

- Analyze the efficacy of Pyr1 in cancer chemotherapy (metastasis, preclinical level)

- Determine prognostic surrogate markers

- Chemical optimization of Pyr1 (stability, solubility)

- Pursuing GLP compliant preclinical development (GRAIN, Start-up “Cellipse”)

Acknowledgements

Biology

Renaud Prudent (IAB, Grenoble)
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Caroline Barette (CMBA, Grenoble)
Catherine Pillet (CMBA, Grenoble)
Emmanuelle Soleilhac (CMBA, Grenoble)
Odile Filhol (INSERM 104, Grenoble)
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Charles Dumontet (INSERM 590, Lyon)
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Ora Bernard (Victoria, Australia)
Stefan Knapp (Oxford, GB)

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Transfert technology office

Emmanuelle Le Coz (FIST, Paris)
Guillaume Rochet (CNRS, Grenoble)
Michel Ferrand (CEA-Grenoble)