

From fundamental research on marine organisms to clinical applications

**Biological mechanisms
& Biomolecules**



Major discoveries made using marine organisms (1)



Sea anemone



phagocytosis



Elie **MECHNIKOFF**
(Nobel 1908)



Squid



Neuronal signal
transmission
in giant axon



Alan **HODGKIN** &
Andrew **HUXLEY**
(Nobel 1963)

Major discoveries made using marine organisms (2)



Fucus algae



fertilization



Gustave Adolphe
THURET (1854)



Hermann **FOL**
(1877)



fertilization,
cell division cycle,
mitosis



Starfish and sea urchin eggs & embryos

Major discoveries made using marine organisms (3)



Cones

conotoxins,
analgesic
drugs
(ziconotide)

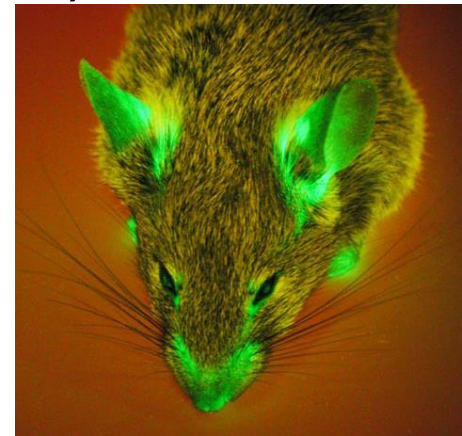
Baldomero **OLIVERA** ('65)
Michael **MCINTOSH** ('80)



Aequorea jellyfish

aequorin,
Green Fluorescent
Protein (**GFP**)

Osumo **SHIMOMURA**
Roger **TSIEN**
Martin **CHALFIE**
(Nobel 2008)



Major discoveries made using marine organisms (4)



Aplysia sea slug

Neuronal connexions in
a ganglion,
Learning & memory

Eric **KANDEL**
(Nobel 2000)



Torpedo ray

Neuronal transmission at the
synapse:
Acetylcholine receptor

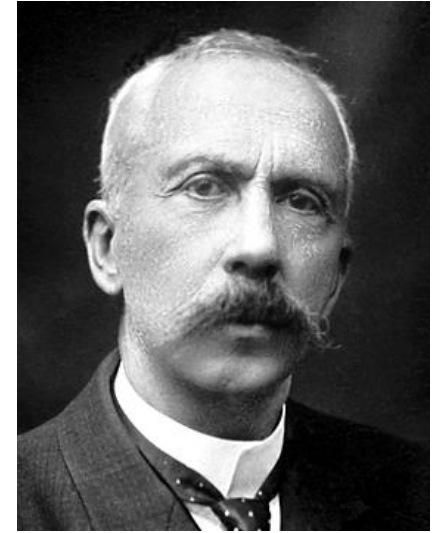
Jean-Pierre
CHANGEUX

Major discoveries made using marine organisms (5)



Physalia physalis Siphonophore

→ Anaphylaxy →



Charles **RICHET**
(Nobel 1913)
Paul **PORTIER**

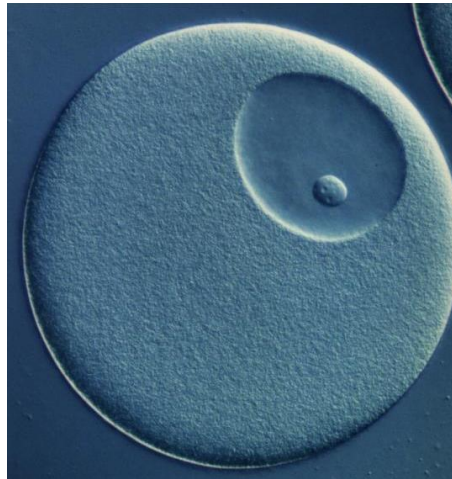


ALBERT I
Prince of Monaco

Major discoveries made using marine organisms (6) (not the same level but same philosophy!)

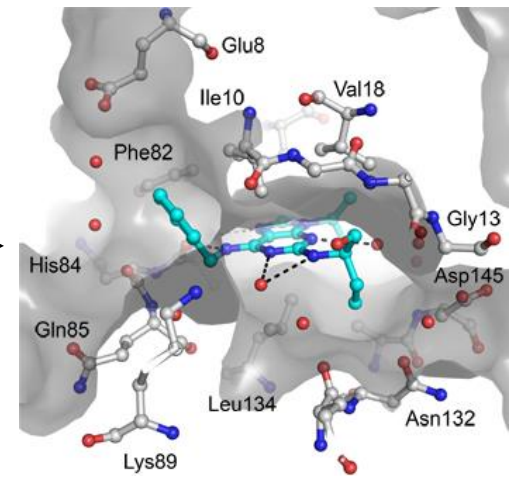


starfish



Oocytes ('78)

- cancers (*clinical*)
- cystic fibrosis (*clinical*)
- rheumatoid arthritis (*clinical*)
- Cushing syndrome (*clinical*)
- glaucoma (*clinical*)
- traumatic brain injury, stroke (*preclinical*)
- glomerulonephritis (*preclinical*)
- polycystic kidney disease (*preclinical*)



CDK1/cyclin B kinase
('82, '85)

Roscovitine ('95),
CR8, finisterines,
perharidines, etc...

Meijer, L., Borgne, A., Mulner, O., Chong, J.P.J., Blow, J.J., Inagaki, N., Inagaki, M., Delcros, J.G. and Moulinoux, J.P., 1997. Biochemical and cellular effects of roscovitine, a potent and selective inhibitor of the cyclin-dependent kinases cdc2, cdk2 and cdk5. **Eur. J. Biochem.** **243**, 527-536.

Meijer, L. and Raymond, E., 2003. Roscovitine and other purines as kinase inhibitors. From starfish oocytes to clinical trials. **Accounts Chem. Res.** **36**, 417-425.

ManRos Therapeutics

“From Sea to Pharmacy”

**Aims: developing drug candidates
in several therapeutic indications**

1. Roscovitine for **Cystic Fibrosis** (CF) with chronic infection
2. Kinase inhibitors for **Polycystic Kidney Disease** (PKD)
3. DYRK1A inhibitors for **Alzheimer's Disease** (AD) & **Down Syndrome** (DS) associated cognitive deficits
4. **Hearing loss** (induced by cisplatin, antibiotics,...) and **stroke**





Isle of Batz

ManRos Therapeutics

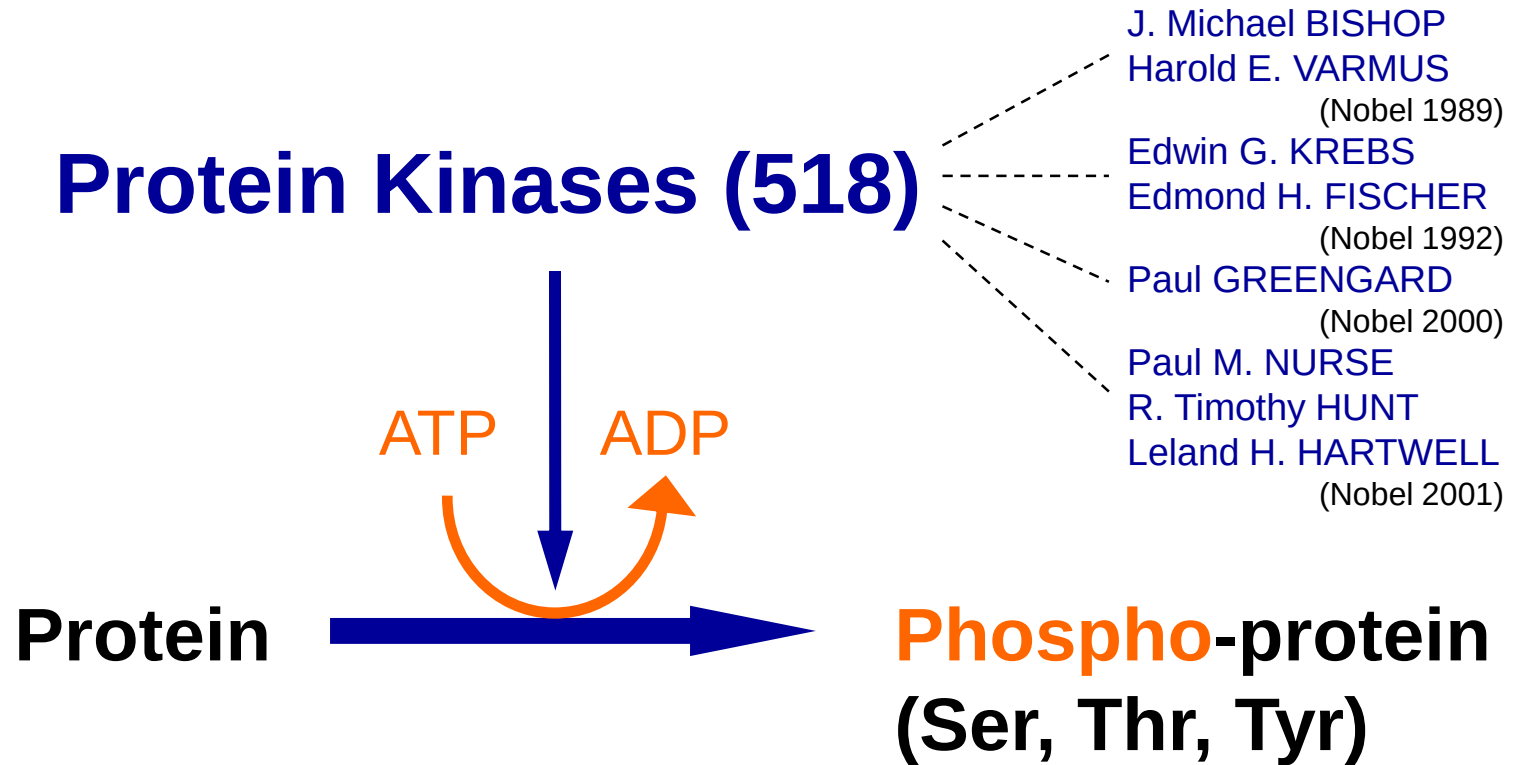
Perharidy peninsula

Ildys Medical Foundation

← Brest 55 km

Roscoff 1 km →

Our expertise: kinases and their inhibitors



- All physiological events are regulated by phosphorylation
- Four Nobel Prizes awarded to the field
- Major human diseases are associated with abnormal phosphorylation
- Kinases = the first therapeutic targets in the Pharmaceutical industry
- 37 kinase inhibitors are now on the market



Edwin **KREBS**

Edmond **FISHER**

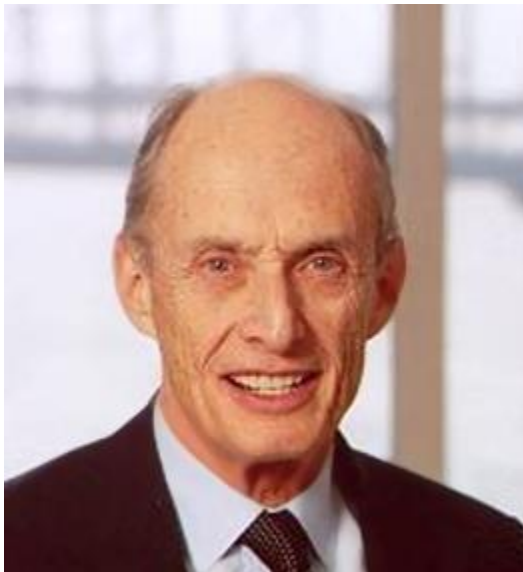
(Nobel 1992)



Paul **NURSE**

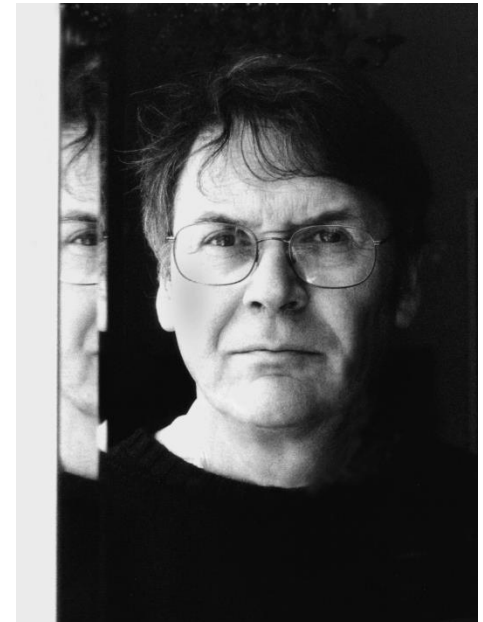
Tim **HUNT**

(Nobel 2001)

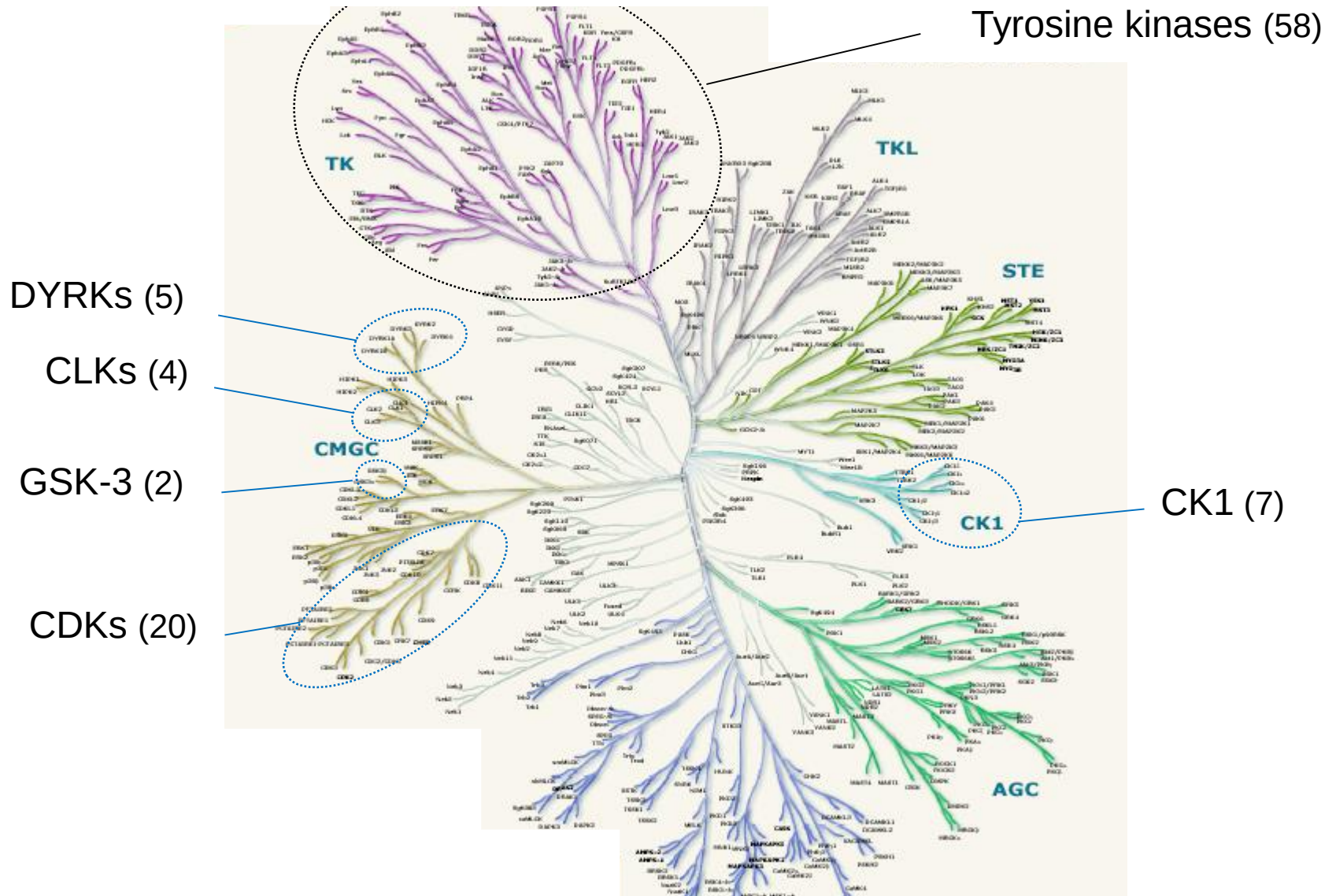


Paul **GREENGARD**

(Nobel 2000)



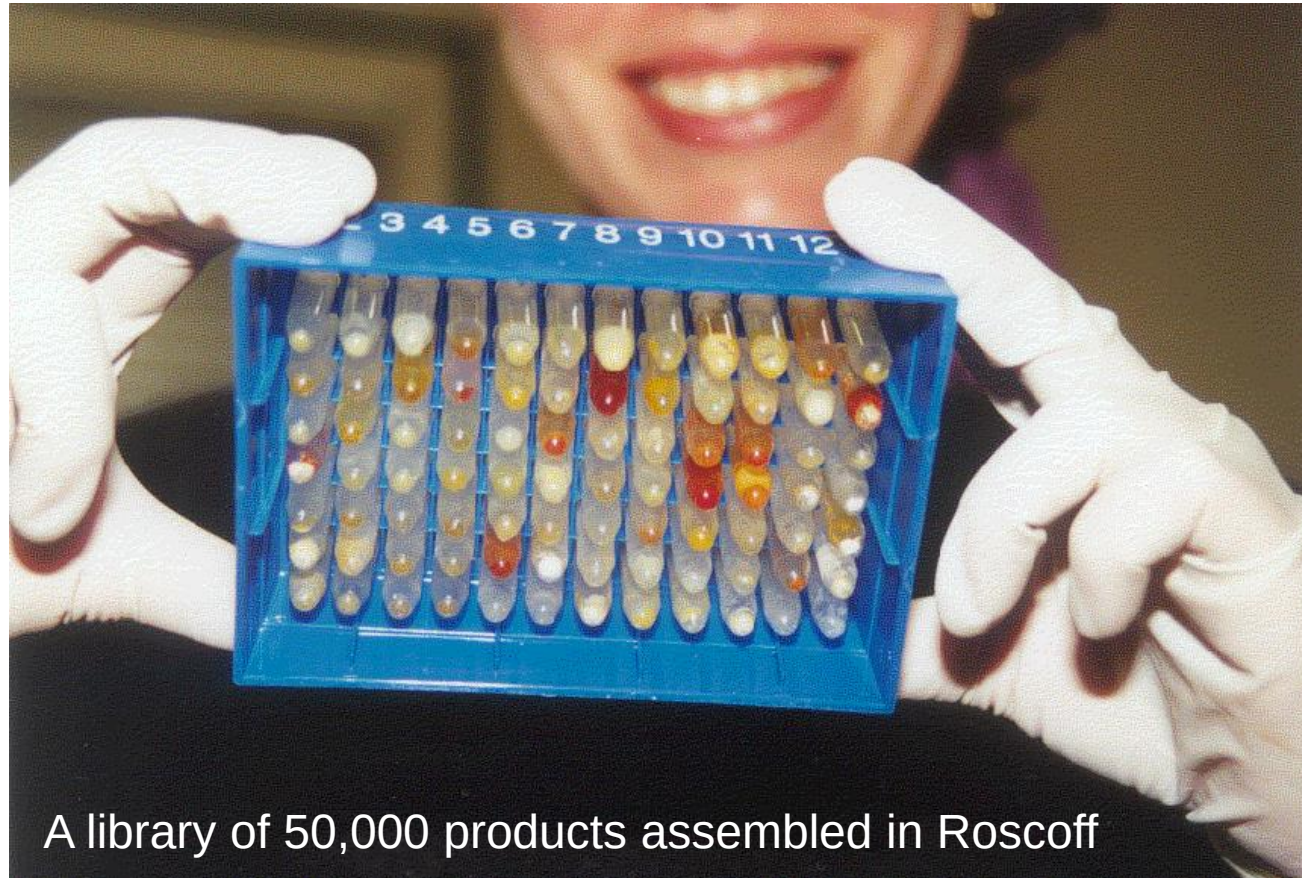
Protein kinases as therapeutic targets (518)



ManRos Therapeutics kinase targets

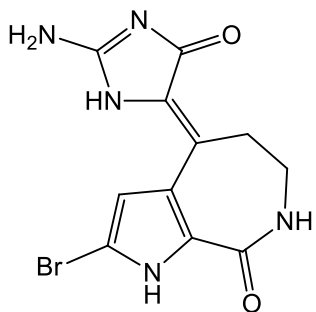
Molecular screening: The search for inhibitory drugs

- libraries of pure synthetic or natural products, or extracts from organisms (bacteria, plants, animals)
- out of 2.000.000 tested molecules, one will become a therapeutic drug...



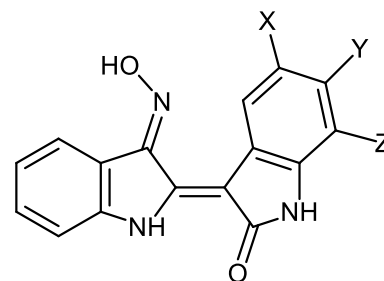
A library of 50,000 products assembled in Roscoff

Hymenialdisine



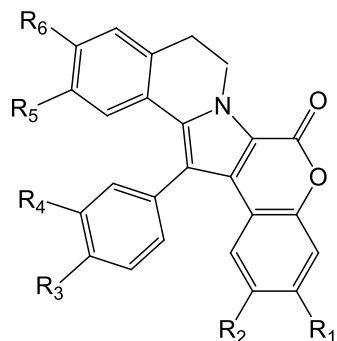
Agelas

Indirubins



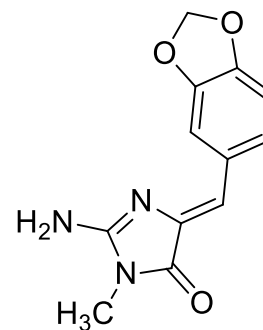
Muricidae & Thaididae

Lamellarins



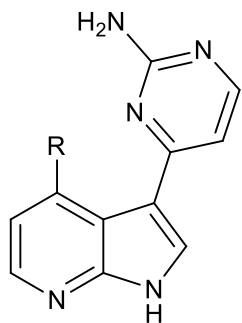
Lamellaria

Leucettines



Leucetta

Meriolins



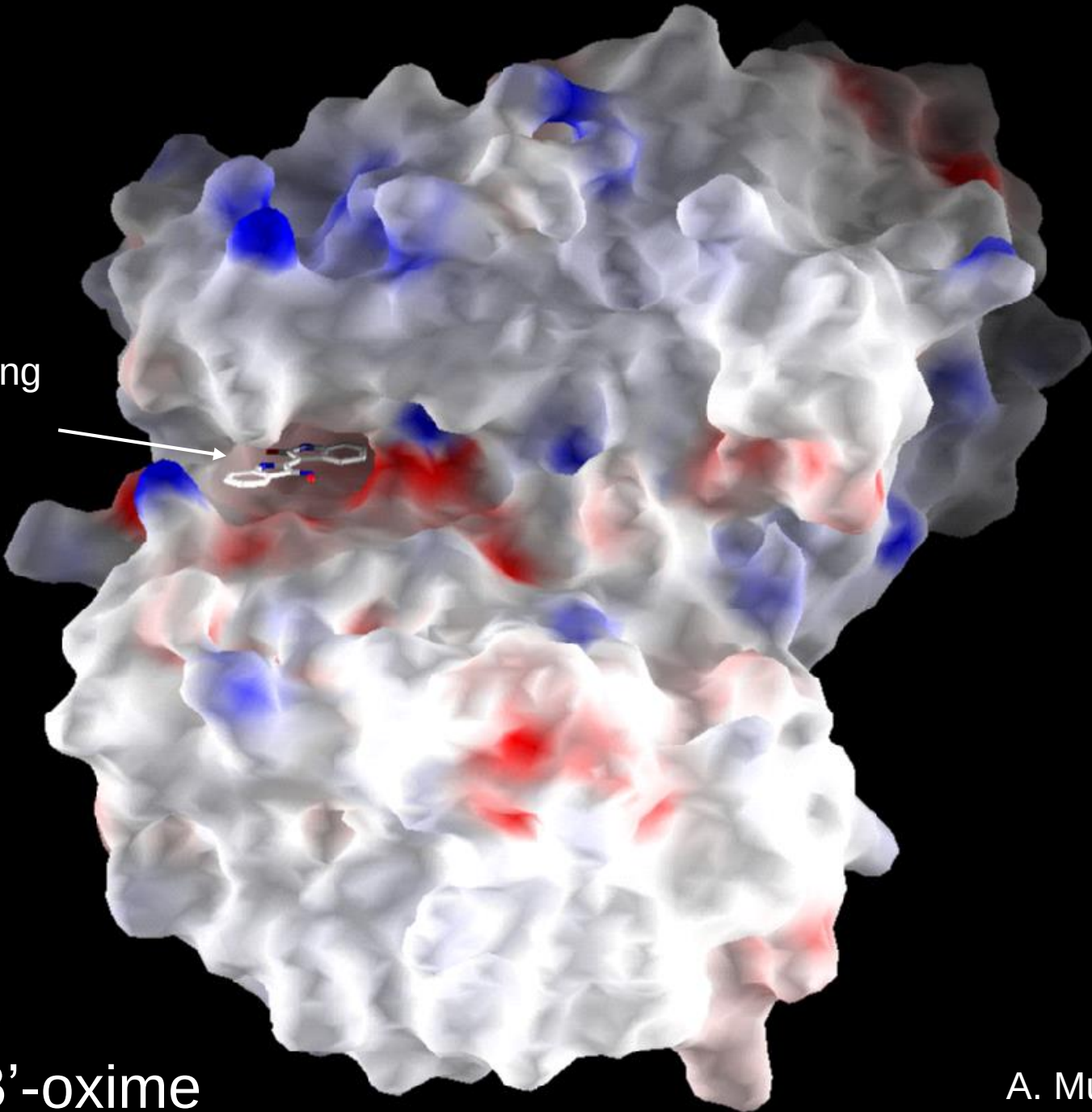
Kirkpatrickia & Aplidium

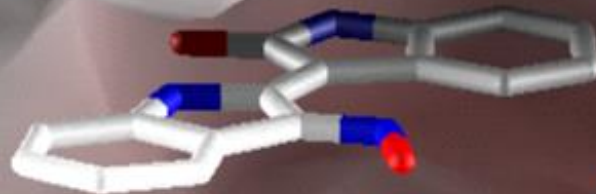
ATP-binding
pocket



CDK5/p25
indirubin-3'-oxime

A. Musacchio





CDK5/p25
indirubin-3'monoxime

CDK inhibitors in non-malignant diseases

Reproduction

- in vitro fertilization
- cloning

Renal diseases

- glomerulonephritis
- lupus nephritis
- collapsing glomerulopathy
- polycystic kidney disease

Obesity

- browning white fat

Viral infections

- HCMV, HSV, HIV, HPV

Protozoan diseases

- malaria
- leishmaniosis
- trypanosomes

Type 2 diabetes

- insulin secretion

Cardiovascular diseases

- atherosclerosis
- restenosis
- cardiac hypertrophy
- arrhythmia
- Timothy syndrome

Inflammation

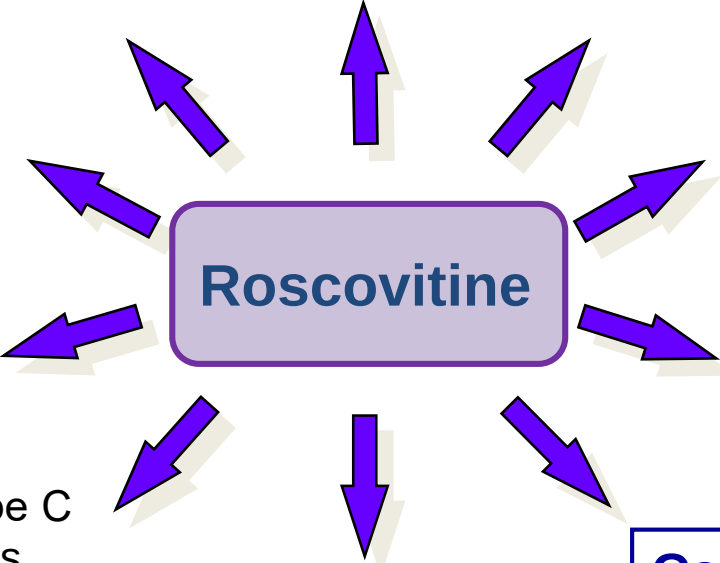
- pleural inflammation
- rheumatoid arthritis
- pneumococcal meningitis
- uveitis

Hearing loss

- noise-, cis-Pt-induced

Cushing's disease

Roscovitine



Cystic fibrosis

- chronic infections & inflammations

Nervous system

- stroke/ischemia
- traumatic brain injury
- Alzheimer's disease
- Parkinson's disease
- Niemann-Pick's disease type C
- amyotrophic lateral sclerosis
- pain signaling

Roscovitine as a drug candidate for chronically infected **Cystic Fibrosis** patients

Targets: CDK2, CDK7, CDK9,
DYRK1A (lymphocytes), TRPC6
(macrophages)

Cystic Fibrosis: scientific and medical context

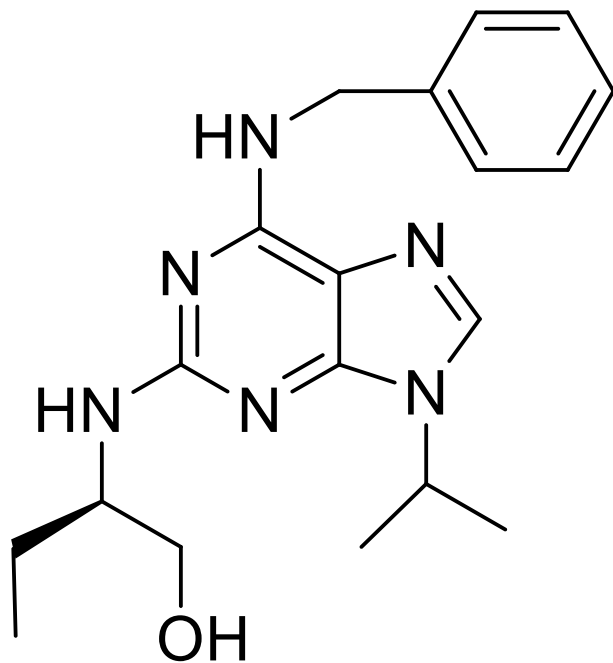
- > Mutations of the **CFTR** gene (*Cystic Fibrosis Transmembrane conductance Regulator*)
- > More than 2,000 mutations described, deletion of Phenylalanine 508 codon (**$\Delta F508$**) represents >70% of all CF cases
- > Respiratory problems and multiple infections by microorganisms (*Pseudomonas aeruginosa*) leading to multi-resistance to antibiotics
- > No treatment until recently: 2013, **Ivacaftor** (Vertex Pharmaceuticals & CF Foundation), corrects G551D-CFTR's activity (3.1% of patients).
2016, **Orkambi** (Vertex)

Number of patients

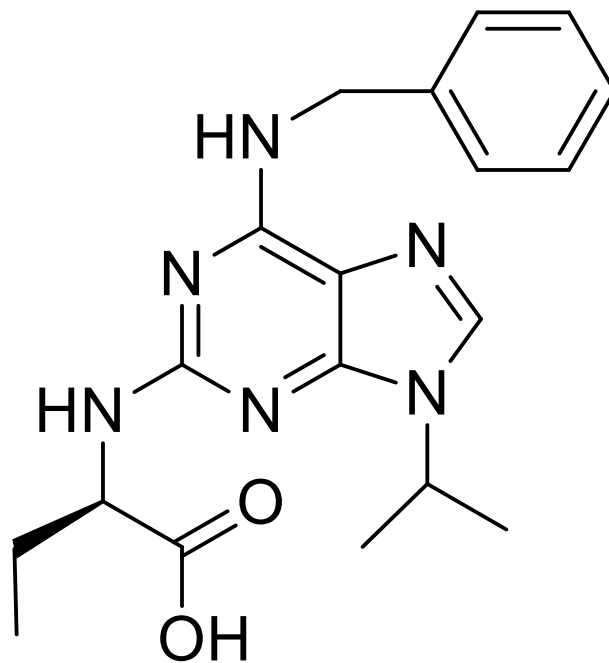
World	70.000
USA	30.000
Europe	35.000
France	6.000



(R)-roscovitrine (Seliciclib), a low molecular weight tri-substituted purine, is a drug candidate for chronically infected **Cystic Fibrosis** patients



(R)-roscovitrine



Metabolite M3

- cheap, fast, optimized 3-step synthesis
- very good oral availability, short half-life, main metabolite is active
- broadly investigated (1,200 papers, >1,000 citations of initial paper, tested over 520 patients)

Roscovitine: main cellular effects

Macrophages

→ acidification of phagolysosomes
→ microbiocidal activity

Neutrophils

→ apoptosis

Eosinophils

→ apoptosis
—| exocytosis

Lymphocytes

—| pro-inflammatory response

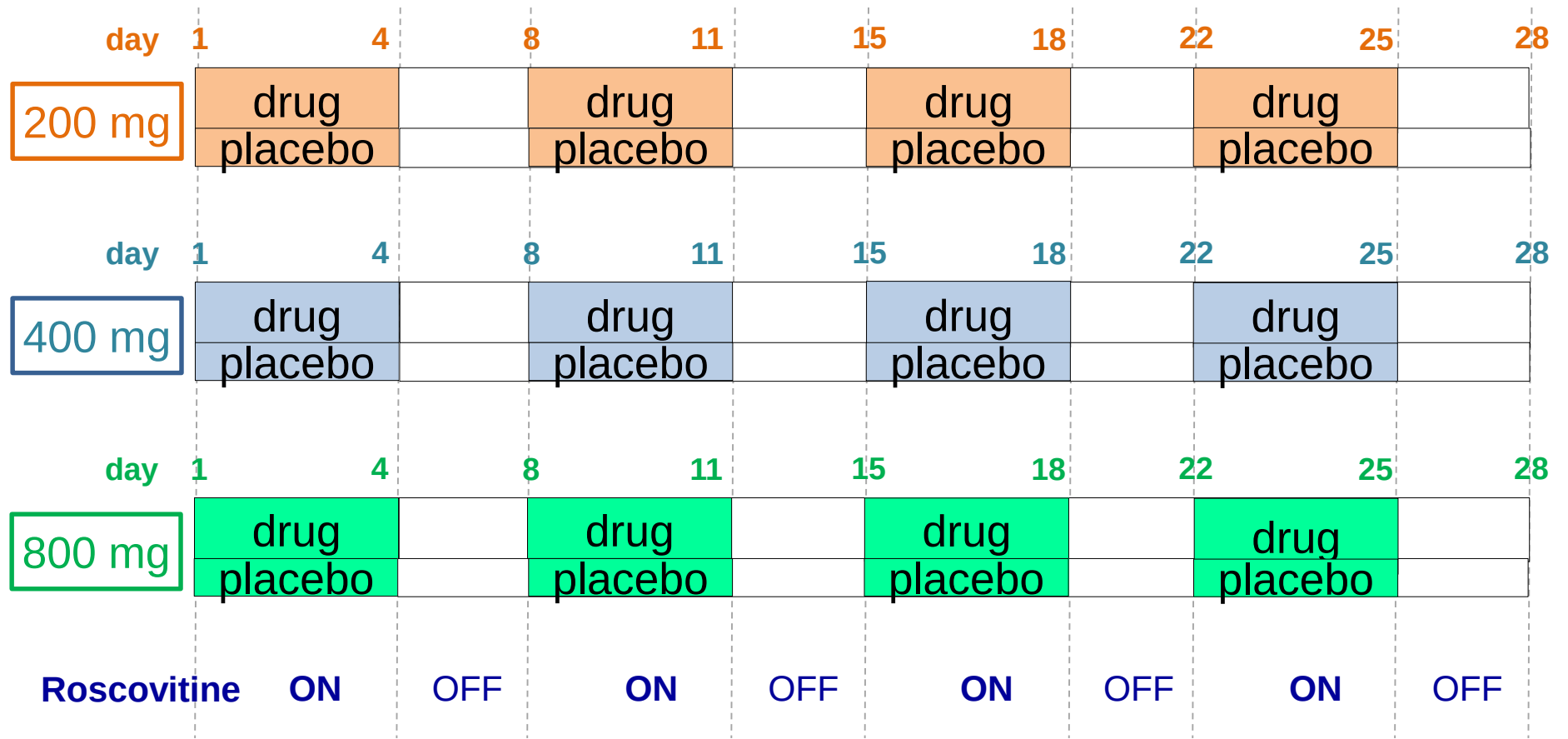
Sensory neurons

→ analgesic effects

Epithelial cells

→ F508del-CFTR trafficking to membrane
—| F508del-CFTR degradation

Essai clinique ROSCO-CF



- 13 hôpitaux en France
- 36 patients, 3 groupes (24 roscovitine/ 12 placebo)
- Objectifs: tolérance & efficacité

**DYRK1A inhibitors as drug
candidates for the treatment of
cognitive deficits
in Alzheimer's disease (AD)
and Down syndrome (DS)**

Targets: DYRK1A, (GSK-3?)

Alzheimer's disease (AD): scientific medical context

World: 25 millions (1 new case / 7 sec)	2050: 115.400.000
USA: 5,3 millions	2050: 13.000.000
Europe: 7,5 millions	2050: 16.510.000
France : 900.000 – 220.000 nouveaux cas /an	2040: 2.200.000

Early-Onset Familial AD (40-50 years) (< 1%) :

Mutations on 3 genes: **APP**, **PS1**, **PS2** → ↑ A β 42/A β 40

Late-Onset AD (>60 years) (> 99%): → ↑ A β 42/A β 40

300 compounds in development

110 clinical trials in progress

4 products on the market

98% clinical failure in the past 20 years...



Down syndrome (DS): scientific and medical context

Down syndrome: no current treatment

World: 6 millions

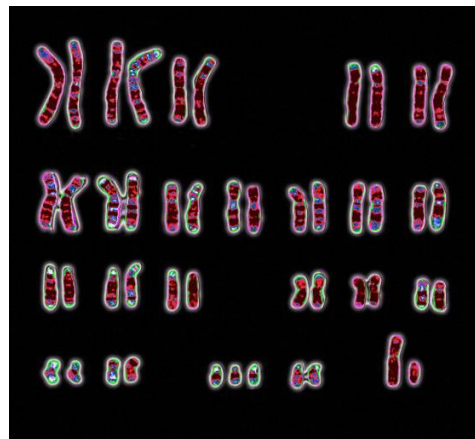
USA: 360,000

Europe: 400,000

France: 65,000

Down syndrome & Alzheimer's disease

- chromosome 21 contains the **DYRK1A**, **BACE2** & **APP** genes, encoding three proteins involved in AD
- **DS recapitulates key aspects of AD:** DS patients develop symptoms of early AD (40 y.) and high frequency of dementia (after 60).
- abnormal **DYRK1A kinase activity** involved in DS & AD **cognitive deficits**
- **inhibiting DYRK1A** should **correct cognitive deficits** of DS & AD



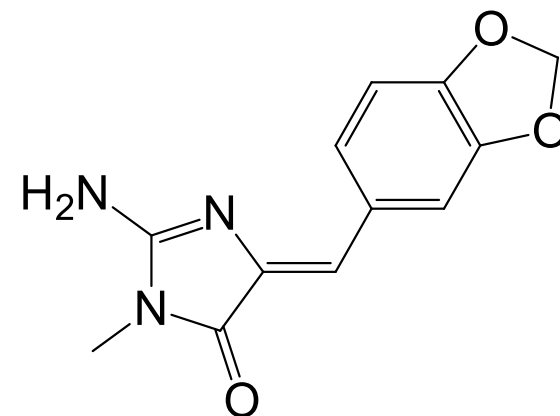
DS karyotype

DYRK1A, Dual specificity, Tyrosine phosphorylation Regulated Kinase 1A
BACE2, β APP Cleaving Enzyme 2
APP, Amyloid Precursor Protein

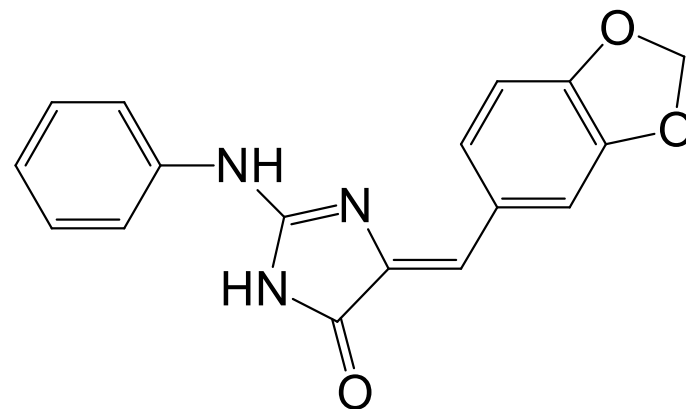
Leucettines, a class of **DYRK1A inhibitors** derived from a marine sponge natural product



Leucetta microraphis (Calcarea)



Leucettamine B



Leucettine **L41**

- Tahtouh, T. et al., 2012. **J. Med. Chem.** **55**, 9312.
Debdab, M. et al., 2011. **J. Med. Chem.** **54**, 4172.
Burgy, G. et al., 2012. **Eur. J. Med. Chem.** **62**, 728.
Fant, X. et al., 2014. **Mol. Pharmacol.** **85**, 441.
Loaëc, N. et al., 2017. **Marine Drugs** **15**, 316.
Nguyen, T.L. et al., 2018. **Disease Models & Mech** **11**, dmm035634.
Souchet, B. et al., 2018. **Brain**, submitted.
Tahtouh, T. et al., 2018. **J. Med. Chem.**, in preparation.

Leucettines prevent cognitive deficits in rodent models of DS and AD (demonstrated in 3 different labs)

Three mouse models of DS

- (a) Tg(Dyrk1a) mice
- (b) Ts65Dn mice
- (c) Dp(16)1Yu mice

Two mouse models of AD

- (a) ICV injected A β 25-35 peptide
- (b) APP/PS1 Δ 29 mice

One rat model of AD

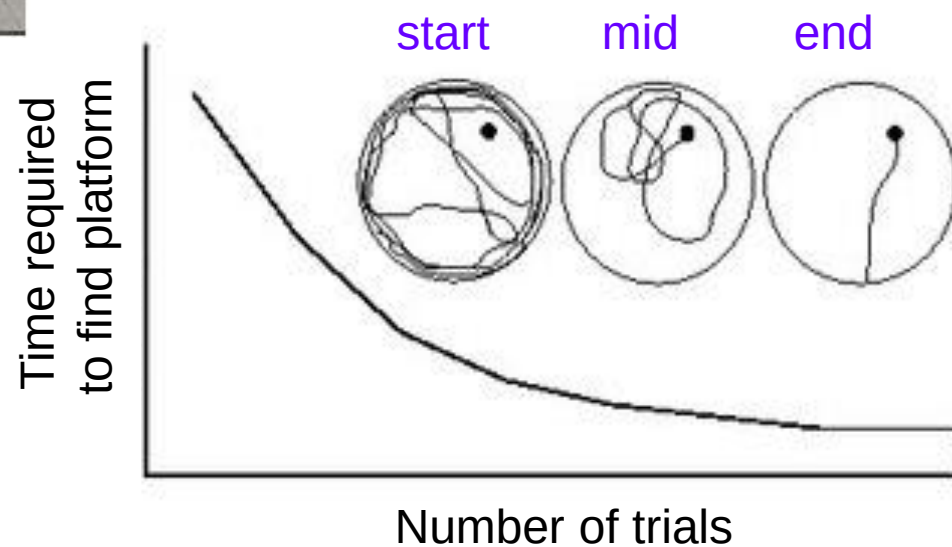
- (a) AAV APP/PS1 model

Naert, G., Ferré, V., Meunier, J., Keller, E., Malmström, S., Givalois, L., Carreaux, F., Bazureau, J.P. and Maurice, T., 2015. Leucettine L41, a DYRK1A-Preferential DYRKs/CLKs Inhibitor, Prevents Memory Impairments and Neurotoxicity Induced by Oligomeric A β 25–35 Peptide Administration in mice. **European Neuropsychopharmacology** 25, 2170–82.

Nguyen, T.L., Duchon, A., Manousopoulou, A., Loaëc, N., Villiers, B., Pani, G., Karatas, M., Mechling, A.E., Harsan, L.A., Limanton, E., Bazureau, J.P., Carreaux, F., Garbis, S.D., Meijer, L. and Héroult, Y., 2018. Correction of cognitive deficits in mouse models of Down syndrome by pharmacological inhibitor of DYRK1A. **Disease Models & Mech.** 11, in press.

Souchet, B., Audrain, M., Billard J.-M., Dairou J., Fol R., Orefice N., Tada S., Dufayet G., Alves S., Potier B., Dutar P., Meijer L., Janel N., Braudeau J. and Cartier N., 2018. Inhibition of DYRK1A proteolysis modifies its kinase specificity and rescues Alzheimer phenotype in APP/PS1 mice. **Brain**, submitted.

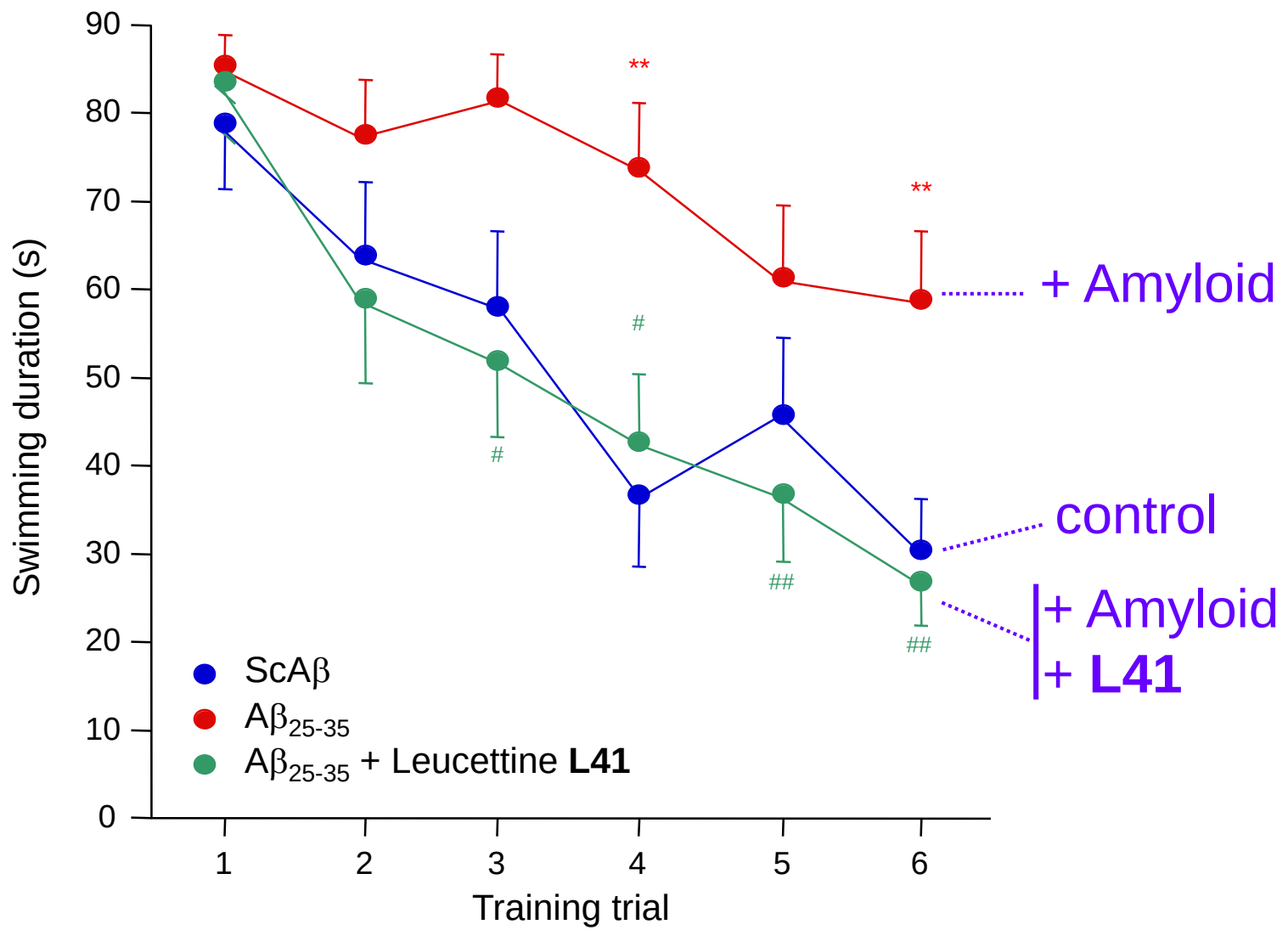
Animal models. Alzheimer's disease models.



Morris water maze

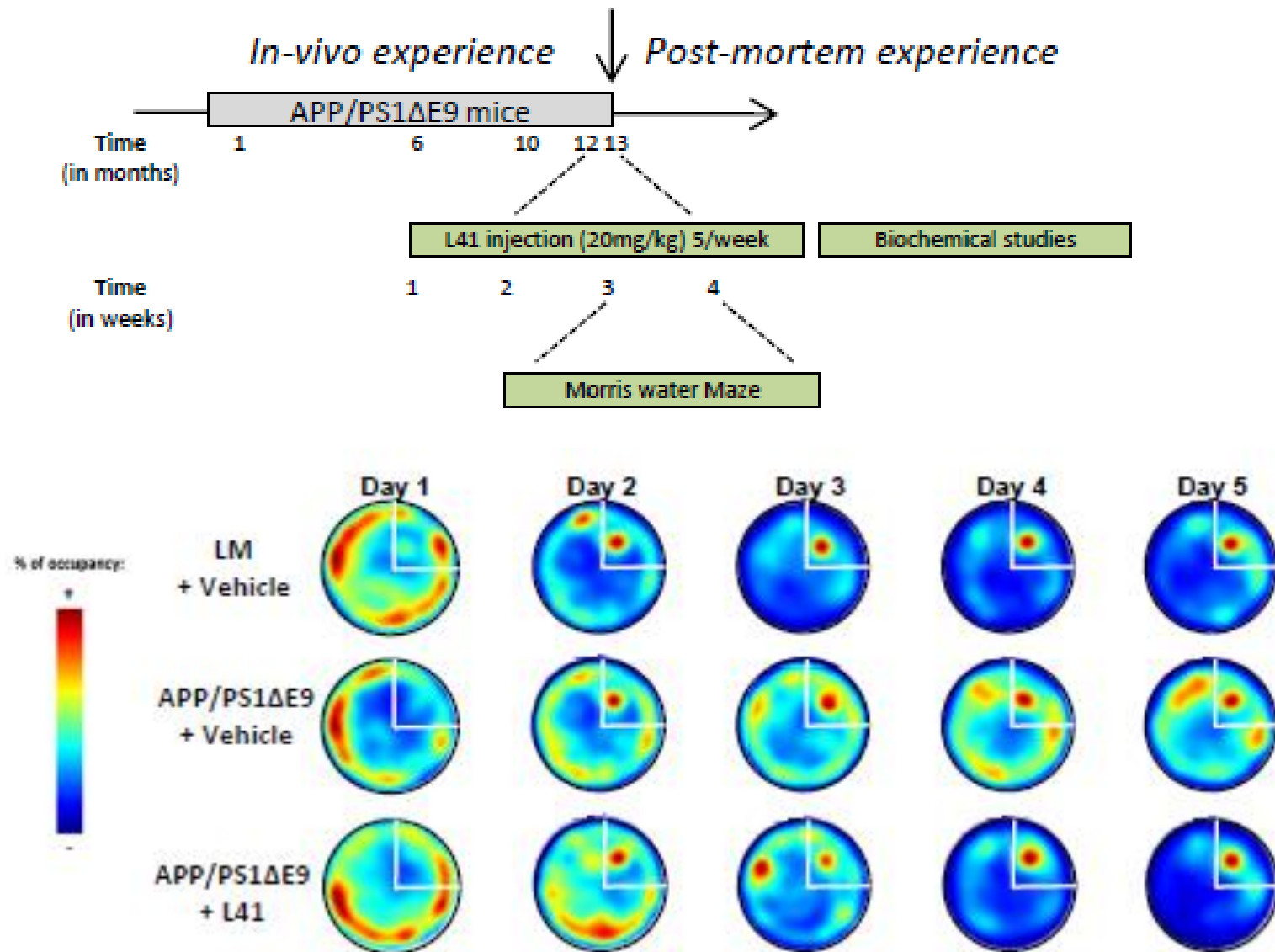
Animal models. Alzheimer's disease models:

(a) ICV injected A β 25-35 peptide

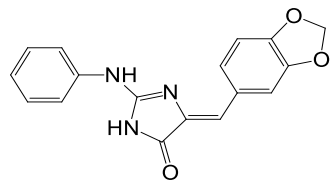


Animal models. 2. Alzheimer's disease model:

(b) APP/PS1 Δ 29 mice



Proof of concept validation. Models & treatments



Leucettine L41

Daily L41, 20mg/kg
or Vehicle treatment (i.p)

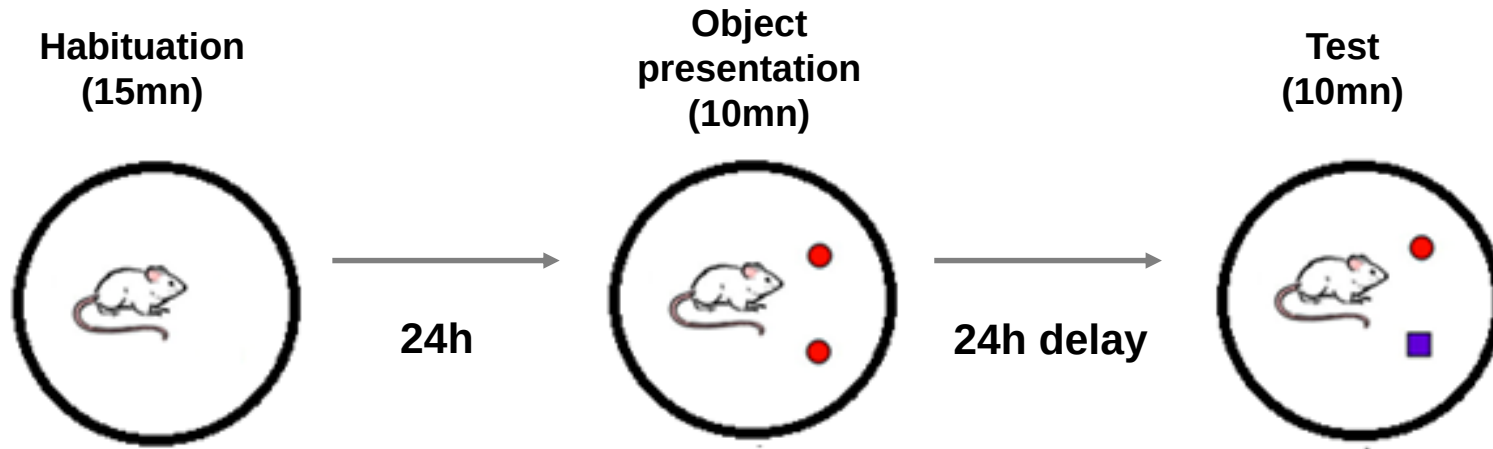


Tg(*Dyrk1a*) and
Ts65Dn

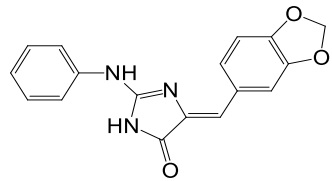


Tg(*Dyrk1a*) or Ts65Dn mice
and respective controls

The Novel Object Recognition test (NOR)



Proof of concept validation. L41 rescues recognition memory of the Tg(*Dyrk1a*) and Ts65Dn mice after 19 days of treatment.



Leucettine L41

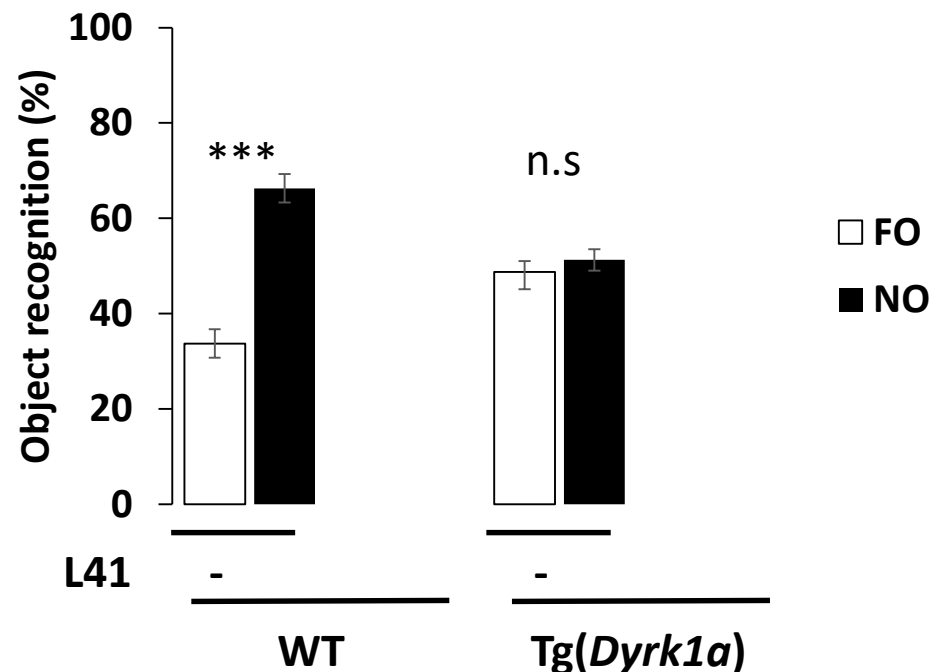
Daily L41, 20mg/kg
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Tg(*Dyrk1a*) and
Ts65Dn



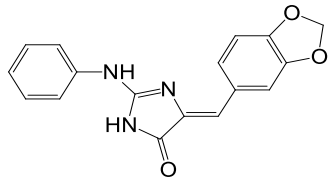
Tg(*Dyrk1a*) or Ts65Dn mice
and respective controls



Statistical analysis: two-way ANOVA test, Tukey post-hoc.

n.s. indicates not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Proof of concept validation. L41 rescues recognition memory of the Tg(Dyrk1a) and Ts65Dn mice after 19 days of treatment.



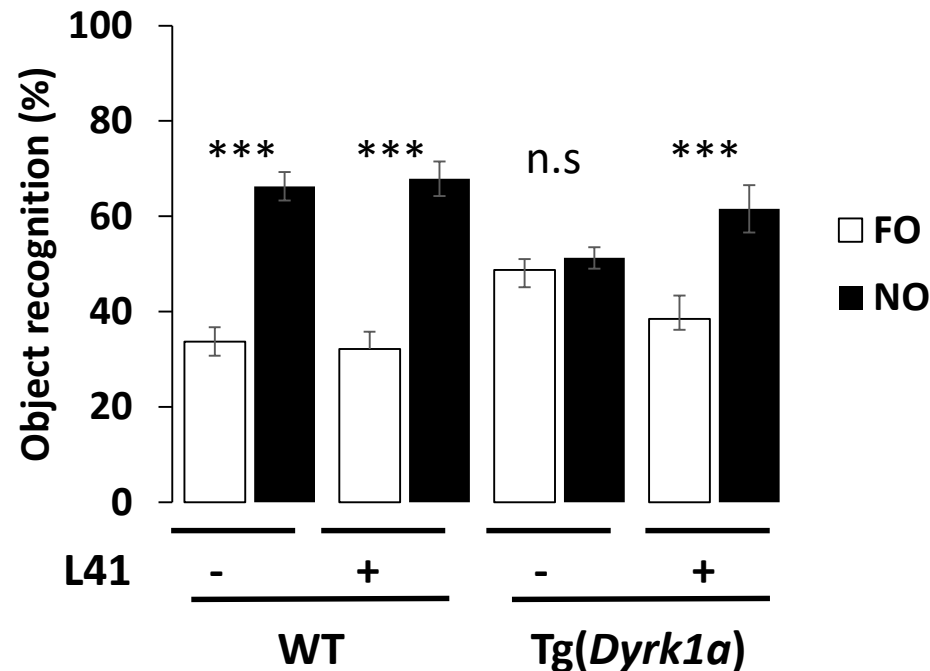
Leucettine L41

Daily L41, 20mg/kg
or Vehicle treatment (i.p)

Tg(*Dyrk1a*) and
Ts65Dn



Tg(*Dyrk1a*) or Ts65Dn mice
and respective controls



Statistical analysis: two-way ANOVA test, Tukey post-hoc.

n.s. indicates not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

An ideal drug candidate

- ☒ potency (1-20 nM IC₅₀)
- ☒ selectivity (DYRK1A, DYRKs, CLKs, ...)
- ☒ no undesired secondary targets
- ☒ oral availability
- ☒ blood/brain barrier permeability
- ☒ pharmacokinetics & biodistribution
- ☒ metabolic stability (metabolism, stable DYRK1A inhibition)
- ☒ efficacy in DS & AD animal models
- ☒ no obvious toxicity
- ☒ intellectual property 2007 (US,EU,JP,CN,RU,MX)

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Yes, but in different Leucettines! (after so many years...)
... and patent getting old...

⇒ **Leucettinibs, a 2nd generation drug family**

ManRos' AD/DS project in summary

Leucetta microraphis (marine calcareous sponge)

2005-2017



Leucettamine B (natural product hit)



Leucettines (synthetic analogs, medicinal chemistry)



Leucettine L41 (Lead 1, biological proof of concept)



Leucettinib L888 (Lead 2, potential drug candidate)



2018-2020

Leucettinib (clinical drug candidate)

meijer@manros-therapeutics.com
06.08.60.58.34



Fonds Baptismaux de la Cathédrale de Laon (12^e siècle)



L'esprit cherche et c'est le coeur qui trouve (G. Sand)
L'échec, c'est un bleu, pas un tatouage!
Tenez-vous loin des gens négatifs; ils ont un problème pour chaque solution (A. Einstein)



études précliniques
(cellules, animaux)

études cliniques
(homme)

mise sur
marché
(AMM)

Drug
discovery

Modèles cellulaire
& animaux

Toxicité
Tolérance

Phase I

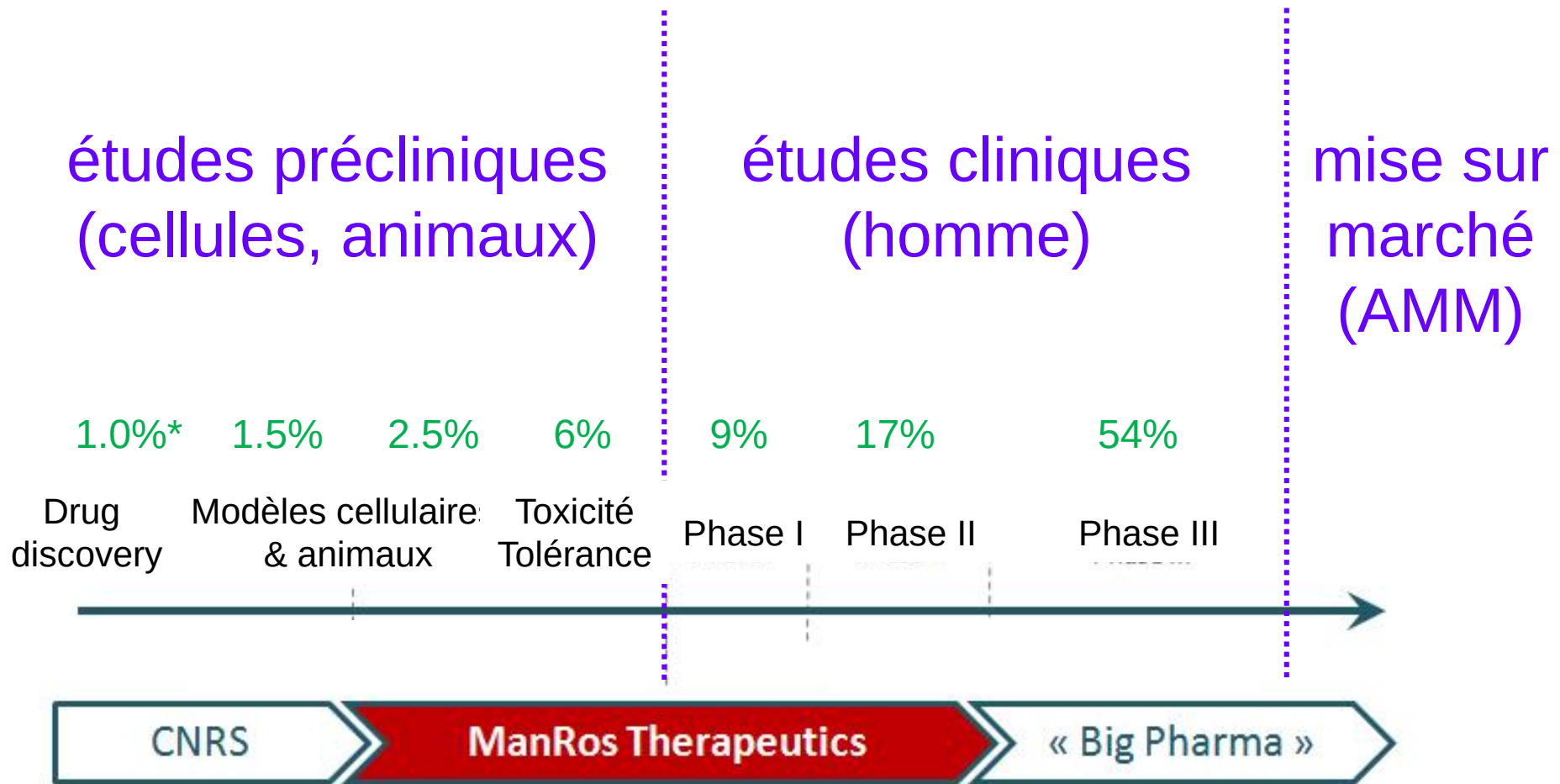
Phase II

Phase III

CNRS

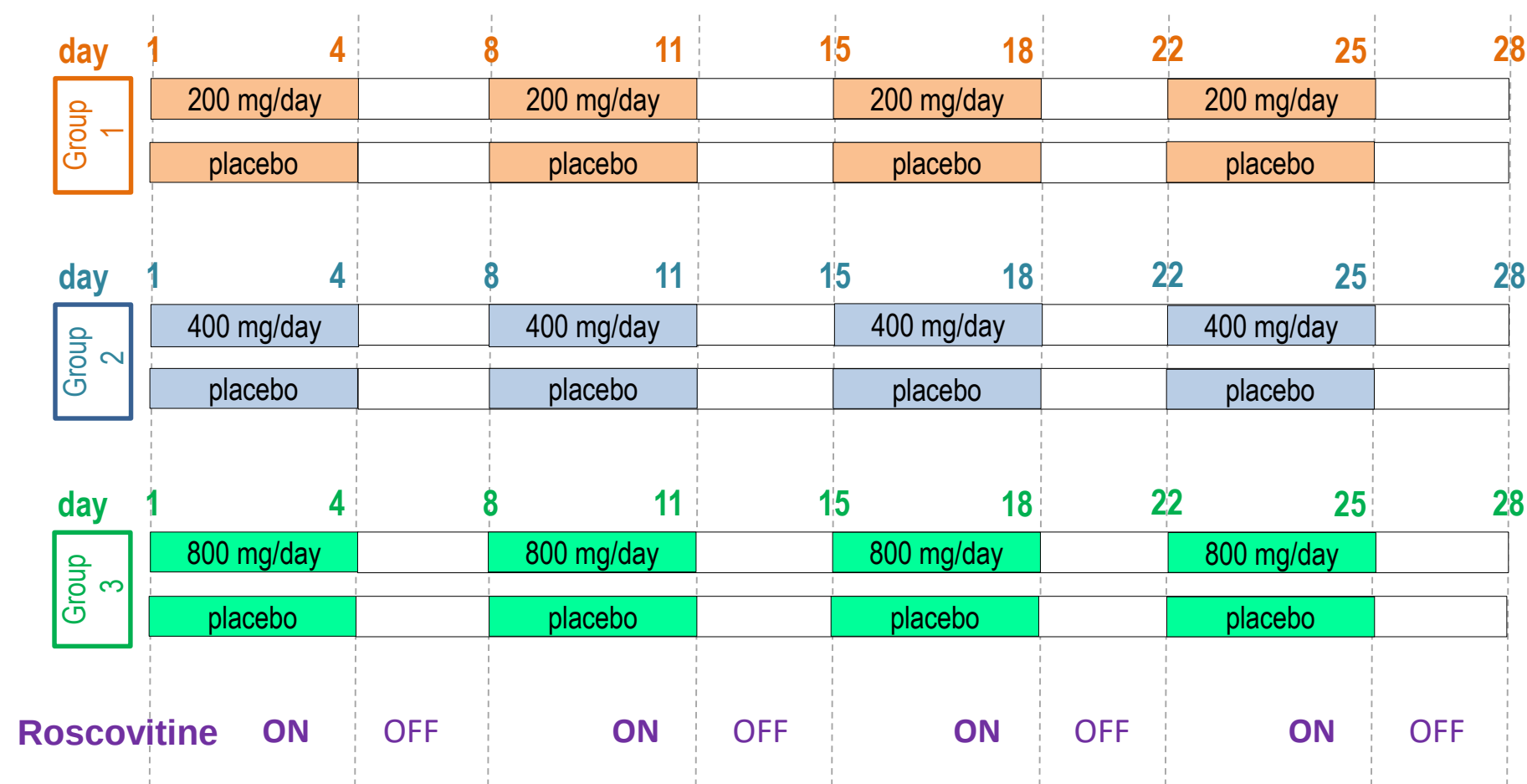
ManRos Therapeutics

« Big Pharma »



Ces % représentent les probabilités d'atteindre l'AMM. Moyennes pour différentes pathologies, elles sont à augmenter pour les maladies rares, les repositionnement de molécules et pour les pathologies sans traitement.

Essai clinique ROSCO-CF



- 13 hôpitaux en France
- 36 patients, 3 groupes (24 roscovitine/ 12 placebo)
- Objectifs: tolérance & efficacité

CDK inhibitors as drug
candidates for the treatment of
**Stroke &
Traumatic Brain Injury (TBI)**

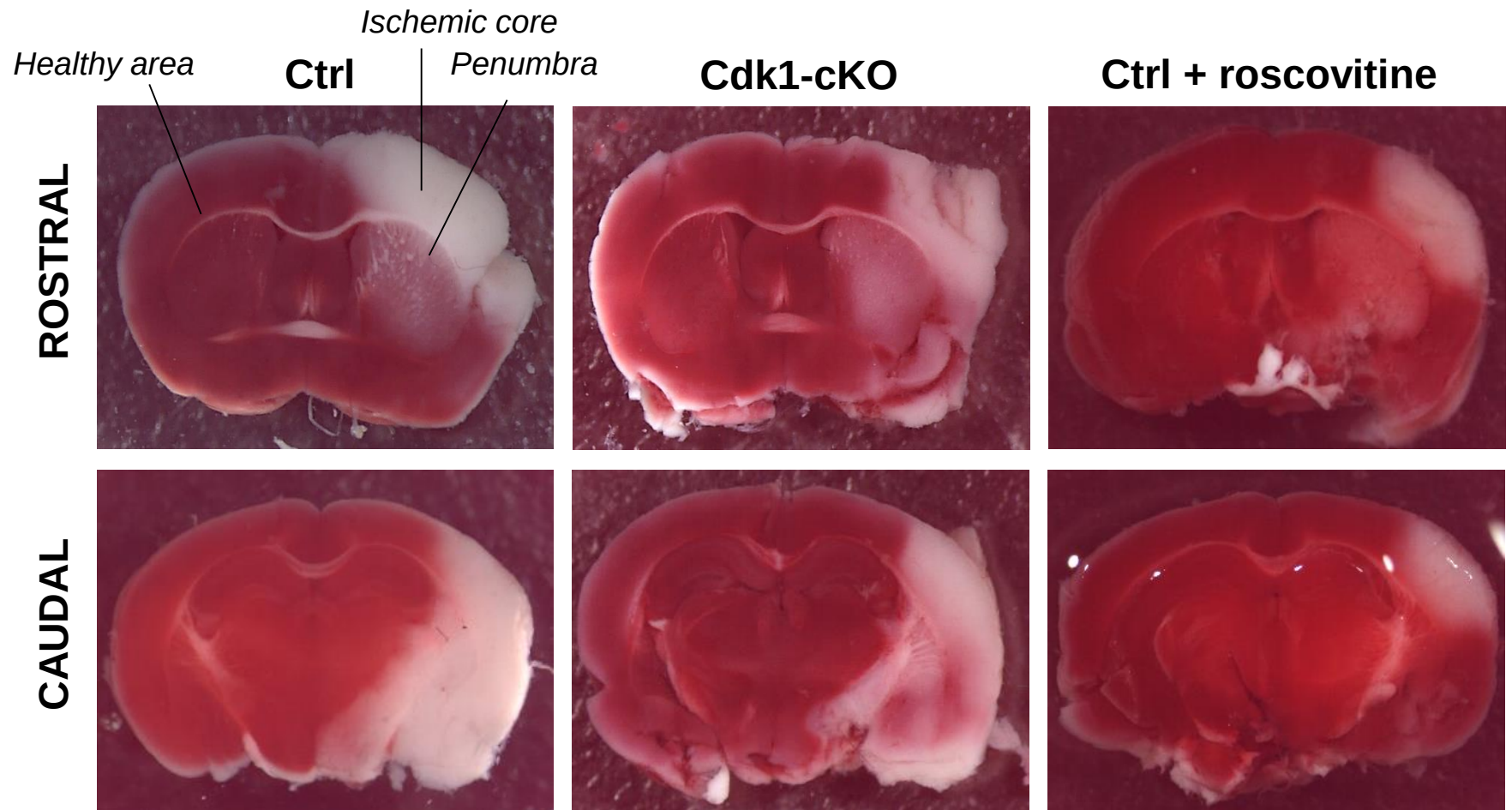
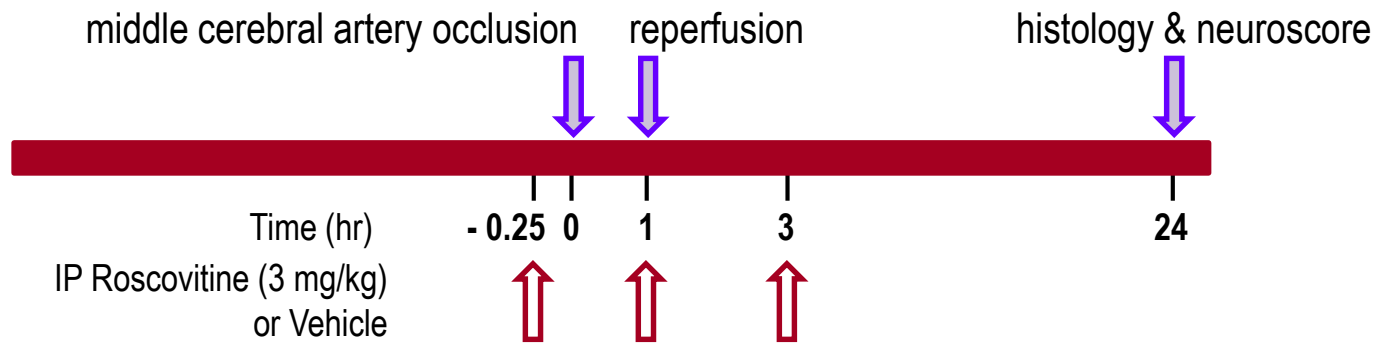
Targets: CDK1, 2, 5

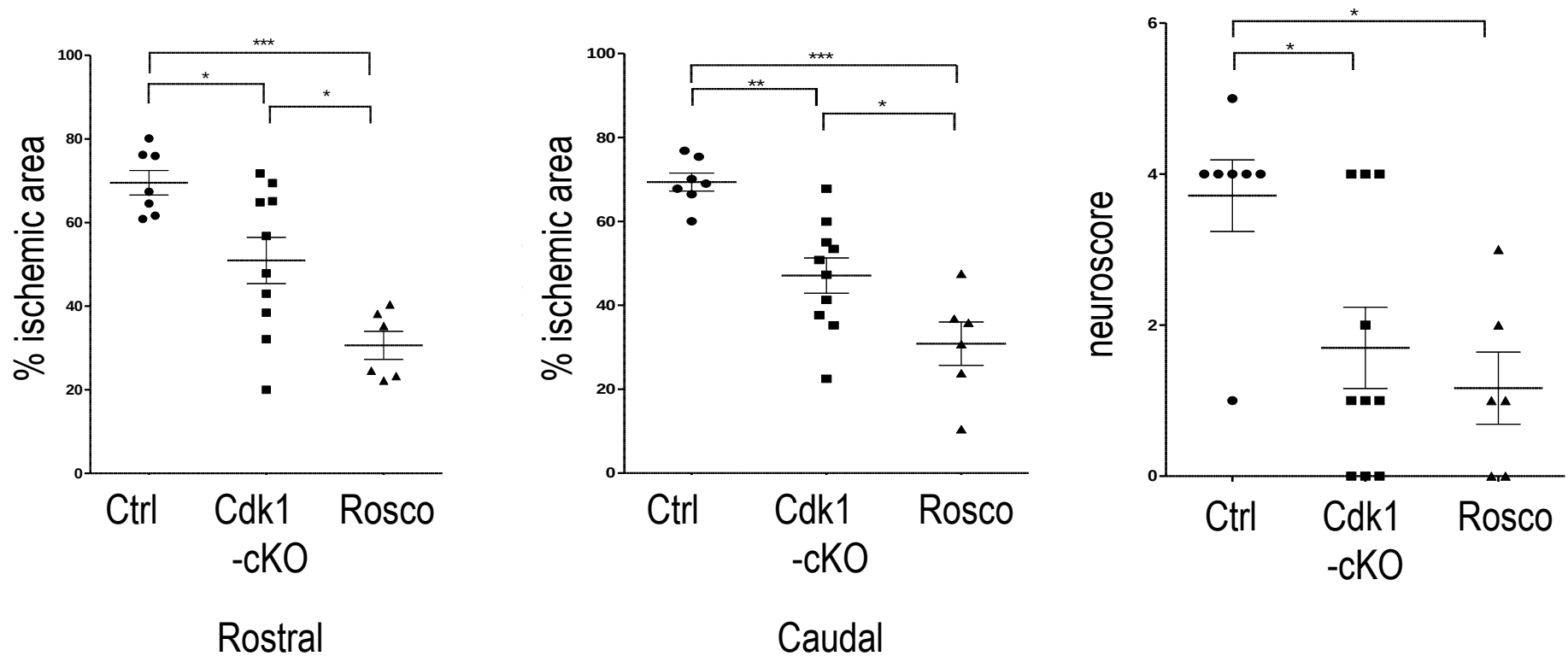
Stroke

Number of patients		
	cases	deaths
World	17.000.000	6.200.000
USA	795.000	140.000
Europe	613.000	110.000
France	142.000	30.000

Traumatic Brain Injury

Number of patients	
World	69.000.000
USA	2.800.000
Europe	1.376.000
France	120.000



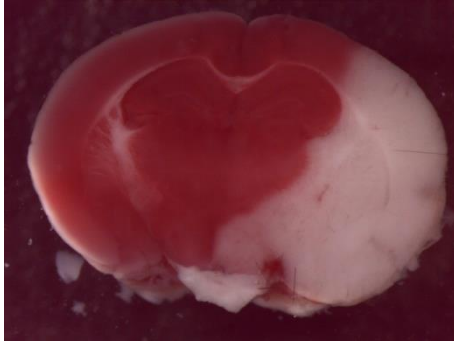


Marlier, Q., Jibassia, F., Verteneuil, S., Linden, J., Kaldis, P., Meijer, L., Nguyen, L., Vandenbosch, R. and Malgrange, B., 2018. Genetic and pharmacological inhibition of Cdk1 provides neuroprotection towards ischemic neuronal death. **Cell Death Discov.** **4**, 43.

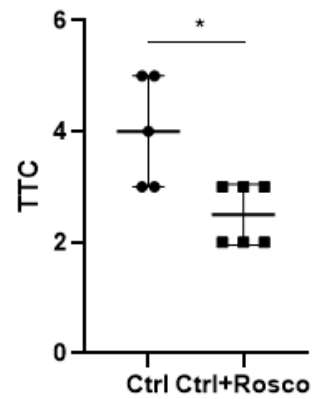
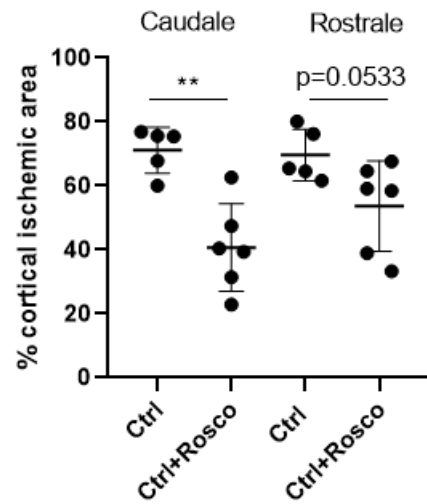
Rousselet E, Létondor A, Menn B, Courbebaisse Y, Quillé ML, Timsit S, 2017. Sustained (S)-roscovitine delivery promotes neuroprotection associated with functional recovery and decrease in brain edema in a randomized blind focal cerebral ischemia study. **J Cereb Blood Flow Metab.**, 271678X17712163.

Menn, B., Bach, B., Blevins T.L., Campbell, M., Ivanov, A., Ben-Ari, Y., Meijer, L., and Timsit, S., 2010. Delayed treatment with systemic (S)-roscovitine provides CDK5-mediated neuroprotection in animal stroke models. **Plos One** **5**, e12117.

ROSTRAL



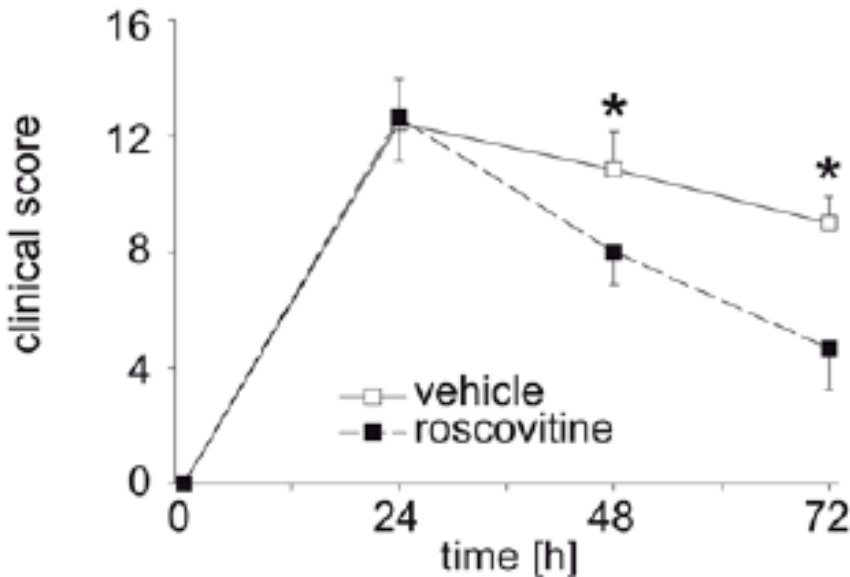
CAUDAL



Resolution of inflammation: pneumococcal meningitis

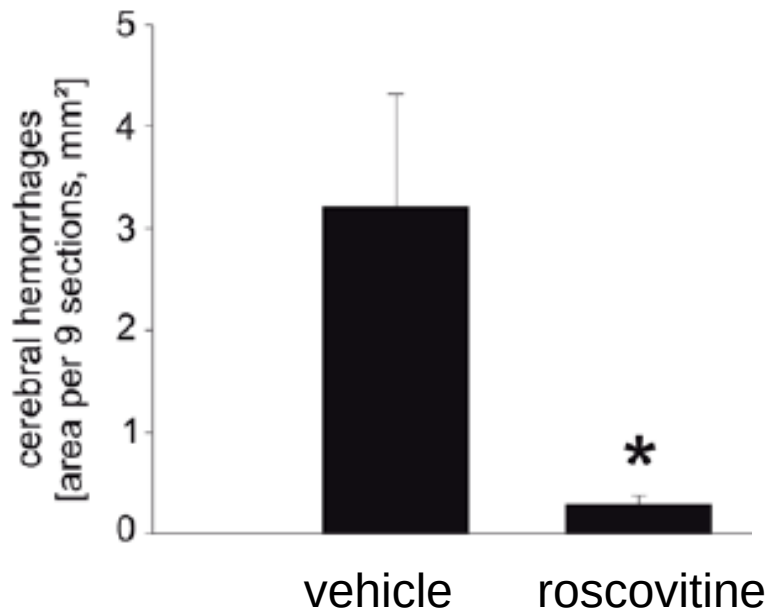
Roscovitone reduces brain tissue damage and accelerates recovery following pneumococcal meningitis

Score scale: from 0 (healthy, uninfected mice to 19 (severe disease and moribund condition) →



vehicle

roscovitone



vehicle

roscovitone

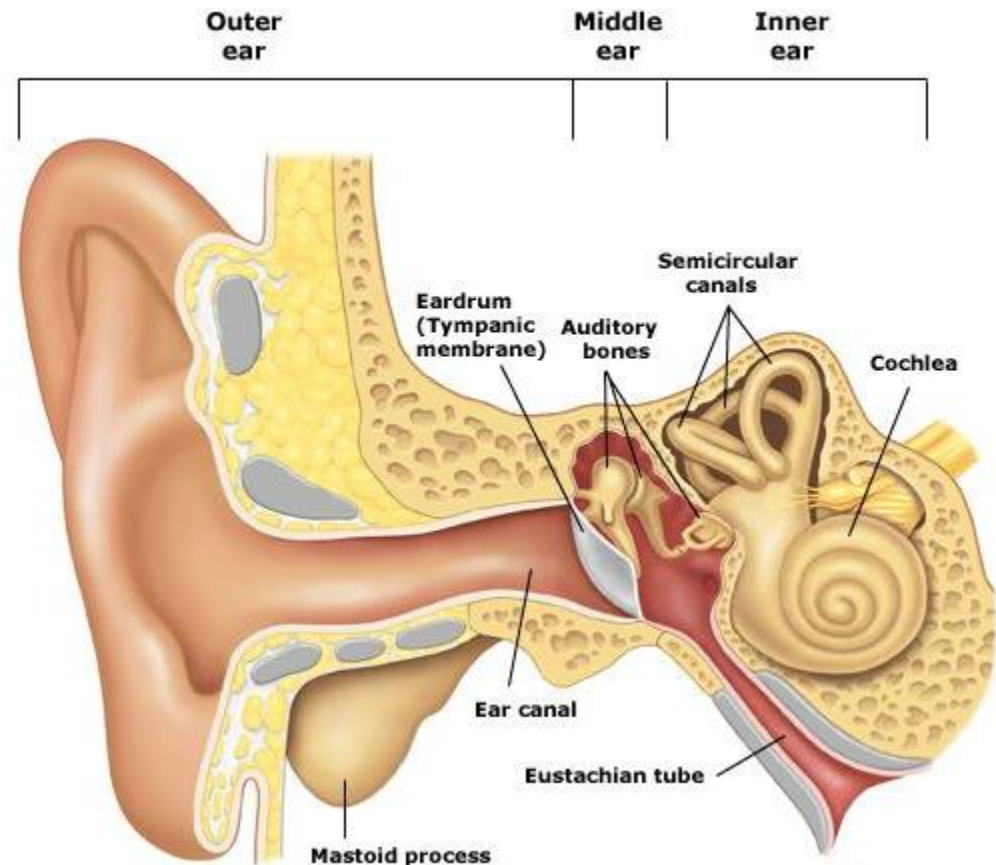
CDK inhibitors as drug candidates for the treatment of noise and cis-Pt induced **hearing loss**

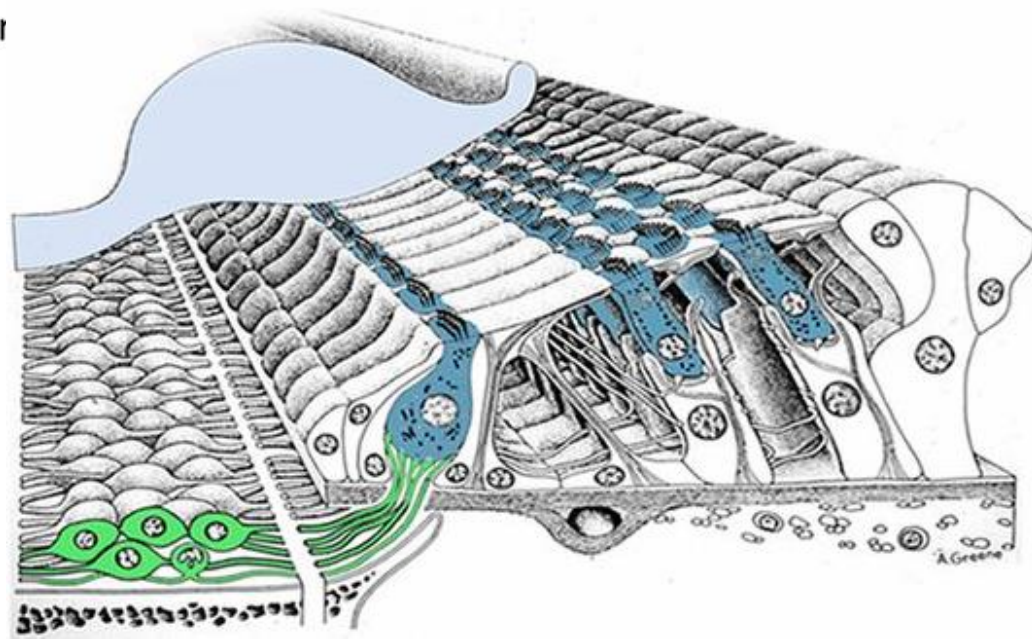
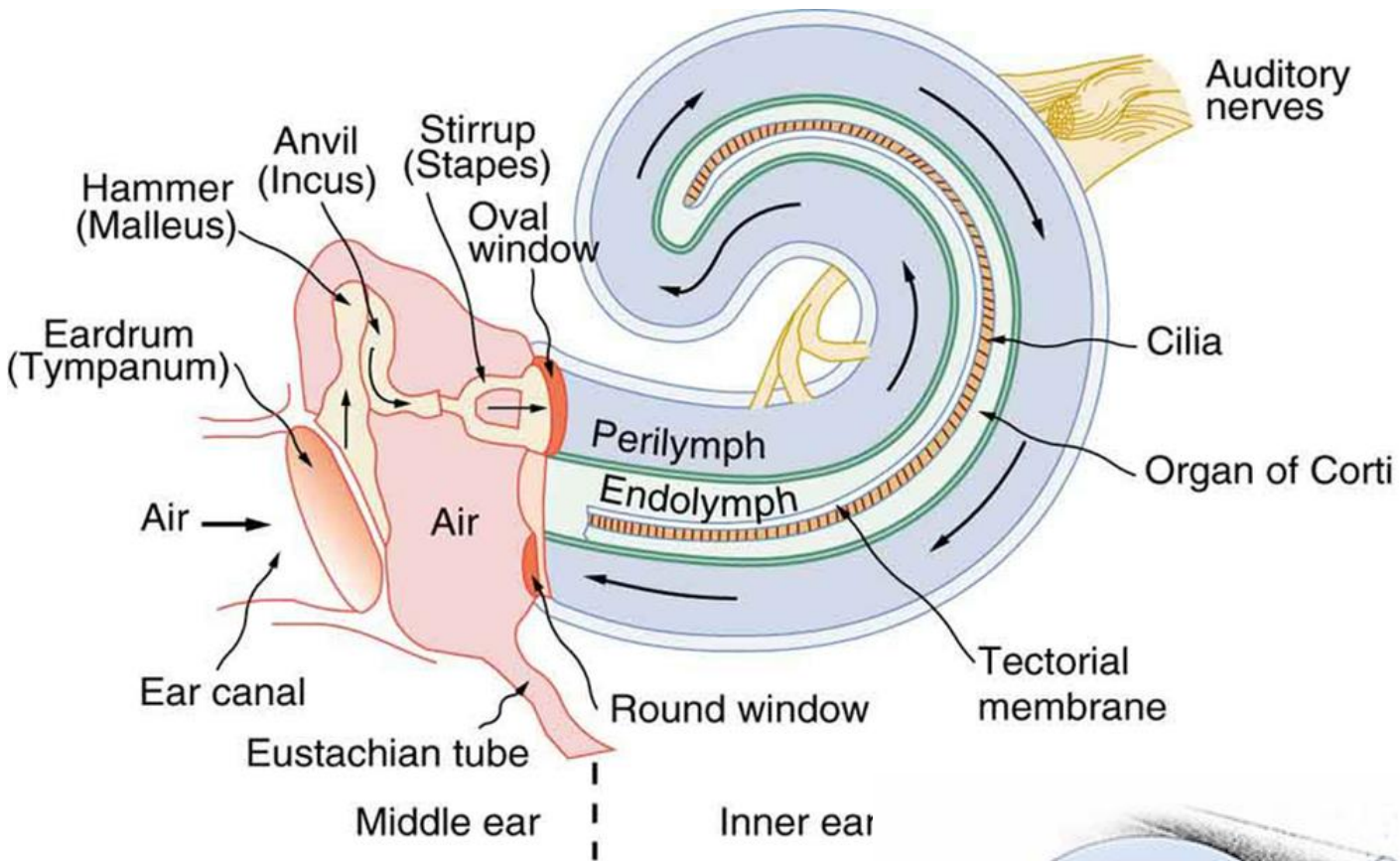
Targets: CDK2 & ?

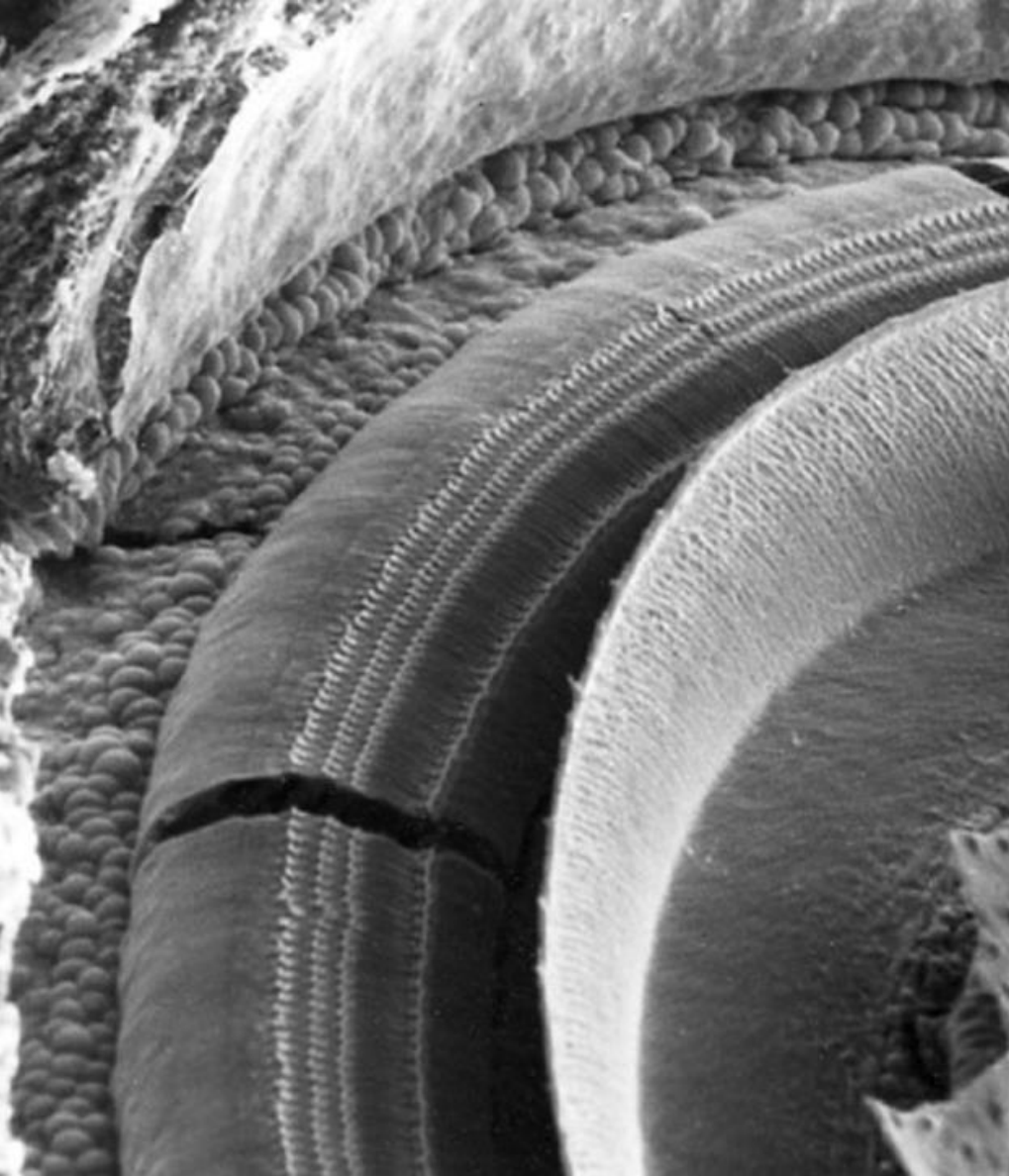
Hearing loss: scientific and medical context

- > Exposure to some antibiotics, some anti-cancer drugs (Cis-Pt), noise (dB). Mutations.
- > Loss of hair cells in the Organ of Corti (12,000 OHC + 3,500 IHC)
- > Serious developmental, societal and economic impact

Number of patients	
World	360.000.000
USA	48.000.000
Europe	51.000.000
France	6.000.000

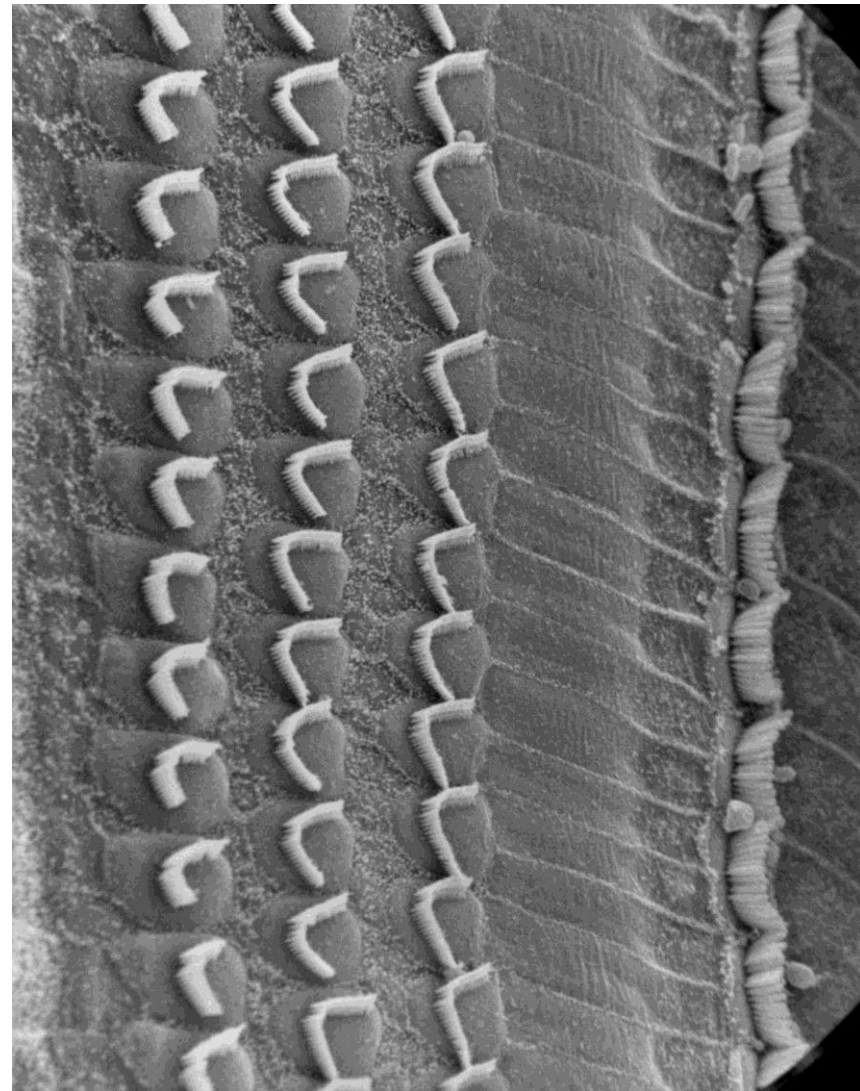




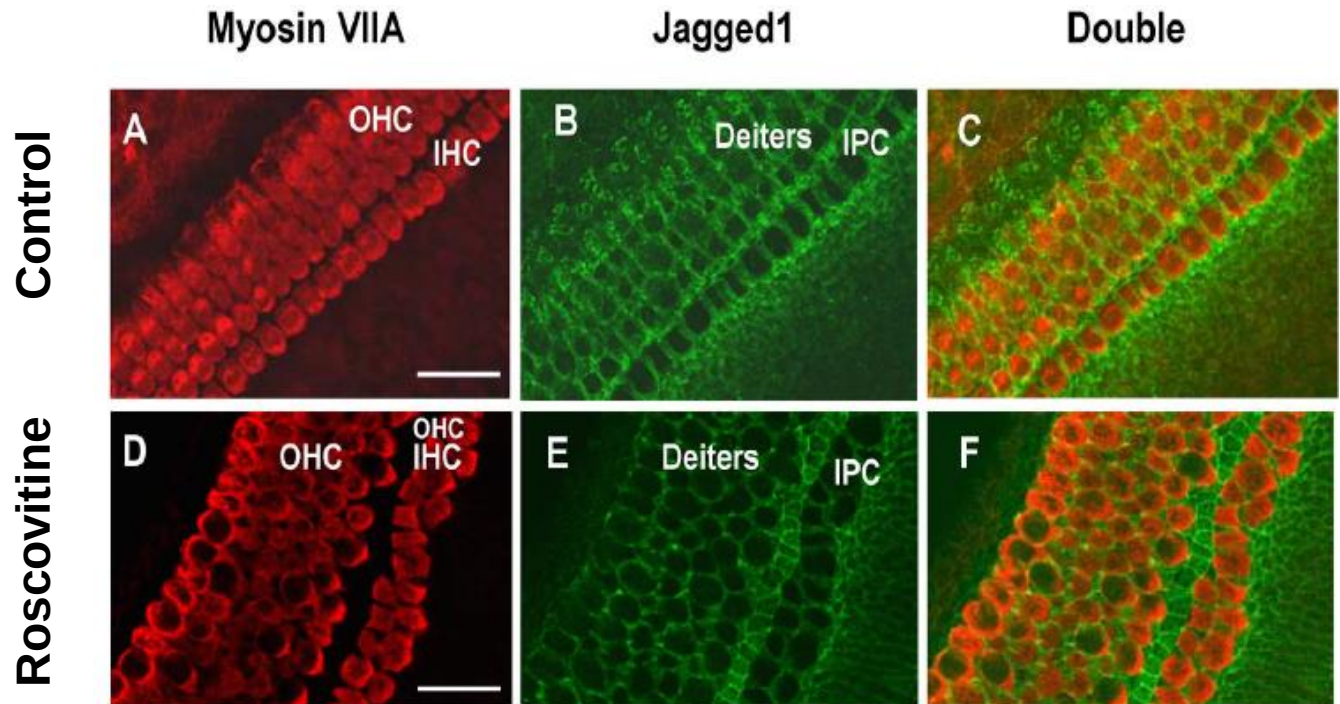
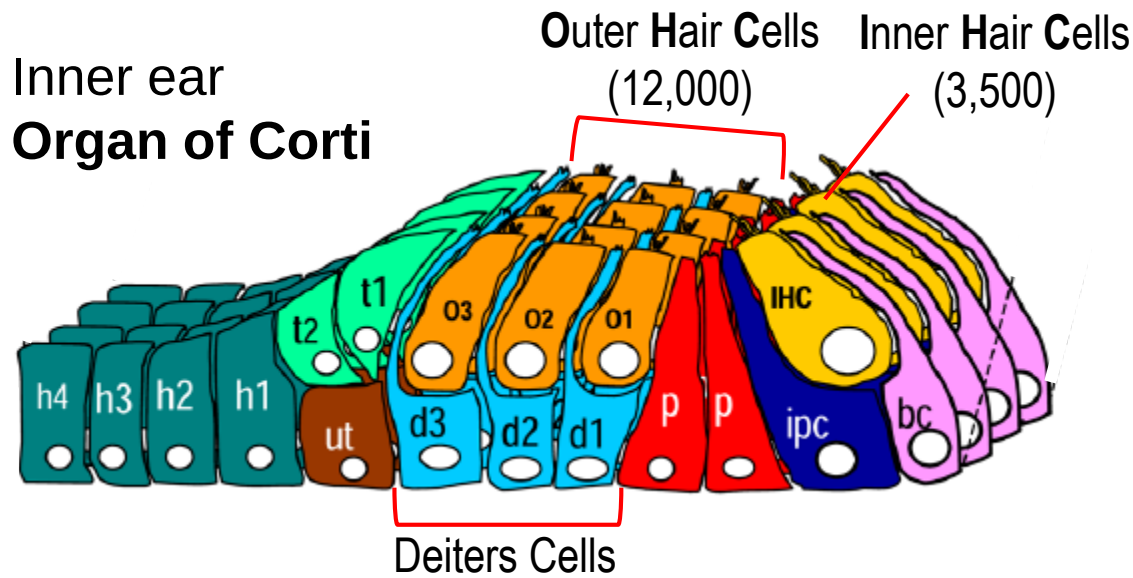


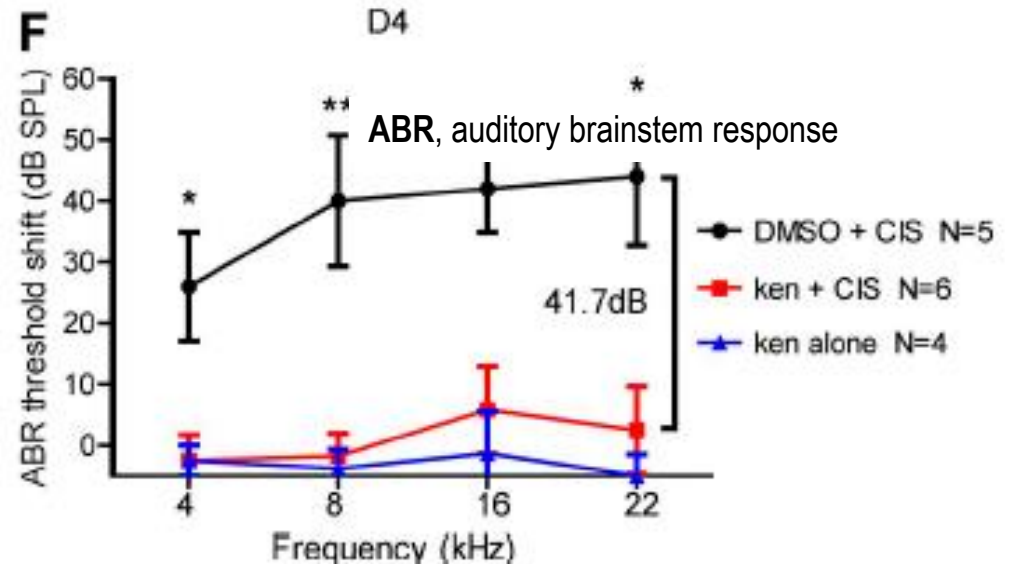
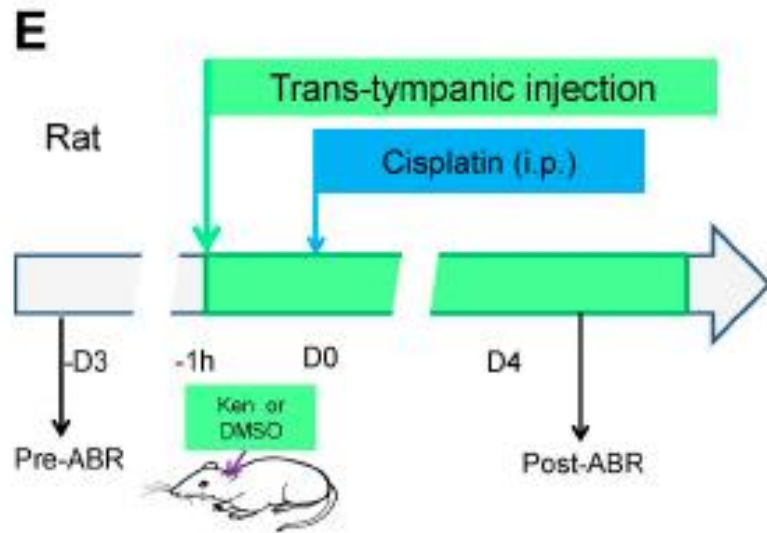
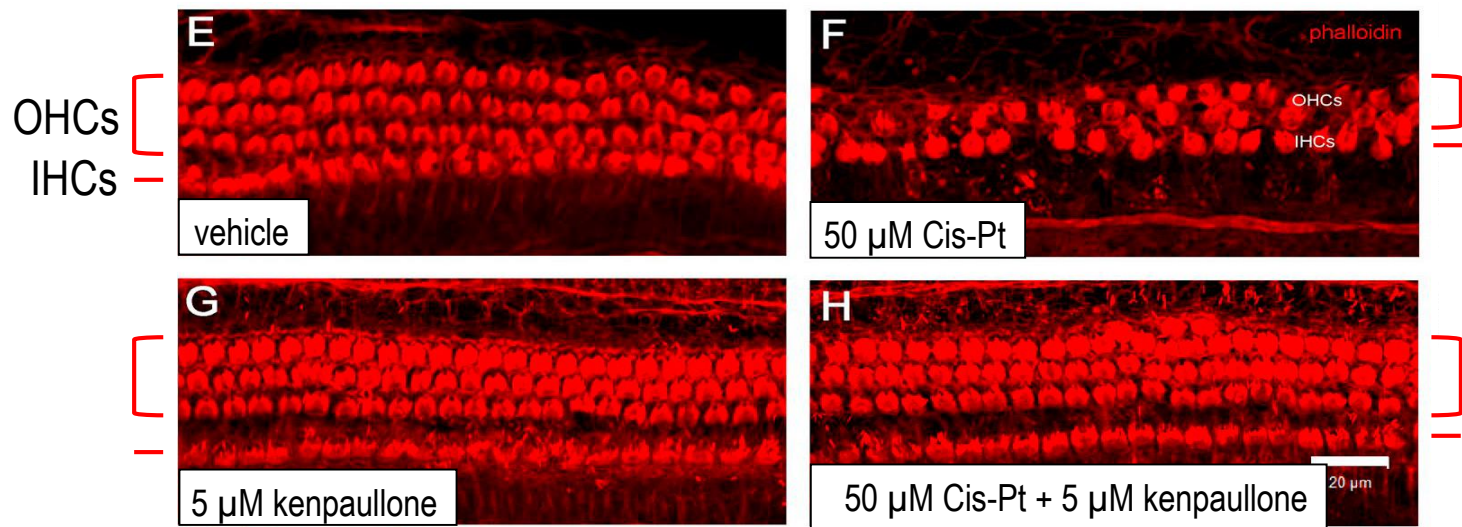
Outer Hair Cells
(12,000)

Inner Hair Cells
(3,500)



Cochlea
Organ of Corti





Teitz T, Fang J, Goktug AN, Bonga JD, Diao S, Hazlitt RA, Iconaru L, Morfouace M, Currier D, Zhou Y, Umans RA, Taylor MR, Cheng C, Min J, Freeman B, Peng J, Roussel MF, Kriwacki R, Guy RK, Chen T, Zuo J, 2018. CDK2 inhibitors as candidate therapeutics for cisplatin- and noise-induced hearing loss. **J Exp Med.** 215, 1187-1203.

Hazlitt RA, Teitz T, Bonga JD, Fang J, Diao S, Iconaru L, Yang L, Goktug AN, Currier DG, Chen T, Rankovic Z, Min J, Zuo J, 2018. Development of Second-Generation CDK2 Inhibitors for the Prevention of Cisplatin-Induced Hearing Loss. **J Med Chem.** 61, 7700-7709.

CDK inhibitors as drug candidates for the treatment of **Polycystic kidney disease**

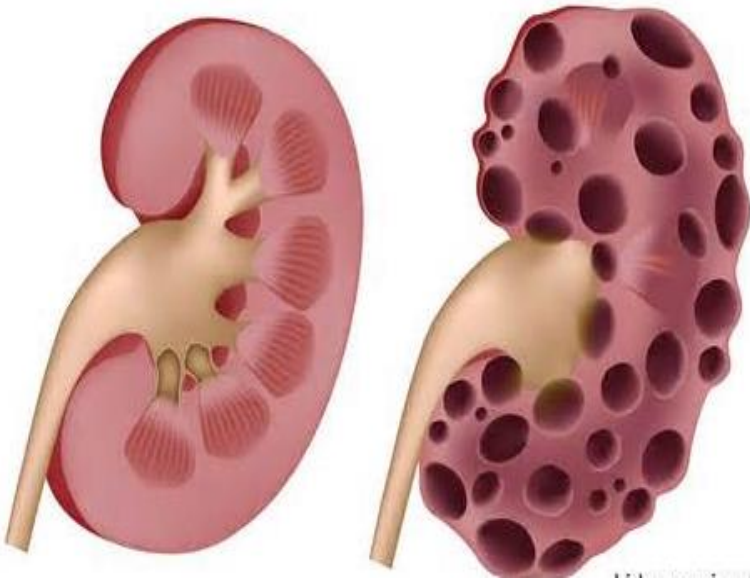
Targets: CDK1, 2, 5, 7, 9,
CK1 α , CK1 ϵ

Polycystic Kidney Disease: scientific medical context

- First genetic disease in terms of prevalence
- Mutations on **PKD1** (85%) or **PKD2** (15%) genes, leading to dysfunctioning of primary cilia (**ciliopathy**)
- Leading to 'End Stage Renal Disease' around the age of 60
- Requires dialysis or renal transplant
- One treatment with modest effects approved in Japan, USA & Europe: **Tolvaptan**

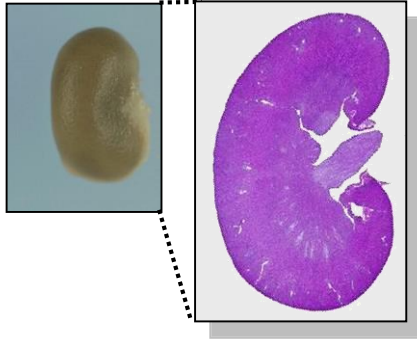
Number of patients

World	12.000.000
USA	648.000
Europe	> 800.000
France	80.000

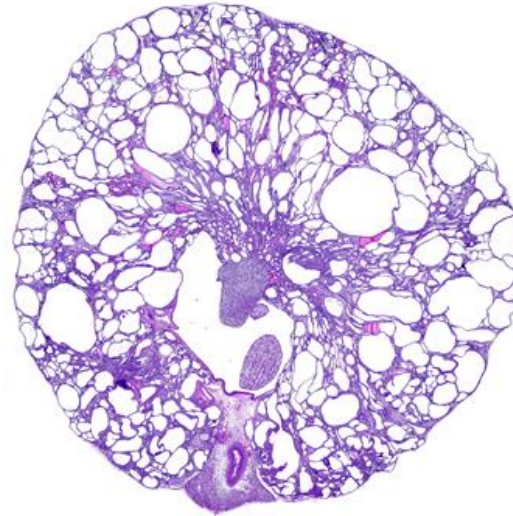
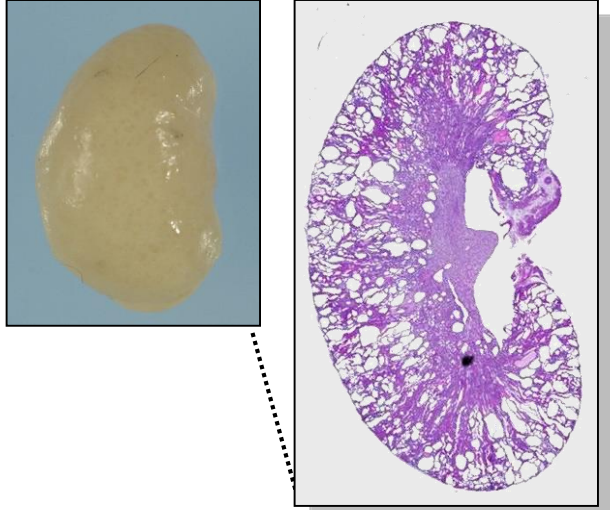


Roscovitine and its derivatives (Finisterines) slow down cystogenesis in PKD models

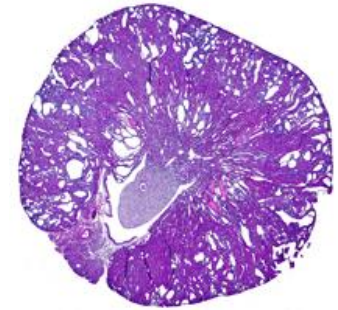
normal 7 wk



cystic 7 wk



vehicle



MRT2-013

Histological section of mice kidneys (jck model)

Bukanov, N.O., Smith, L.A., Klinger, K.W., Ledbetter, S.R. and Ibraghimov-Beskrovnaya, O., 2006. Long-lasting arrest of murine polycystic kidney disease with CDK inhibitor Roscovitine. **Nature** **444**, 949-952.

Bukanov, N.O., Moreno, S.E., Natoli, T.A., Rogers, K.A., Smith, L.A., Ledbetter, S.R., Oumata, N., Galons, Meijer, L. and Ibraghimov-Beskrovnaya, O., 2012. CDK inhibitors R-roscovitine and S-CR8 effectively inhibit renal and hepatic cystogenesis in an orthologous model of ADPKD. **Cell Cycle** **11**, 4040-4046.