Leucettinib-21, a **DYRK1A** kinase inhibitor drug candidate aiming at correcting **cognitive disorders** in people with **Alzheimer's disease** or **Down syndrome**

Perha
Pharmaceuticals

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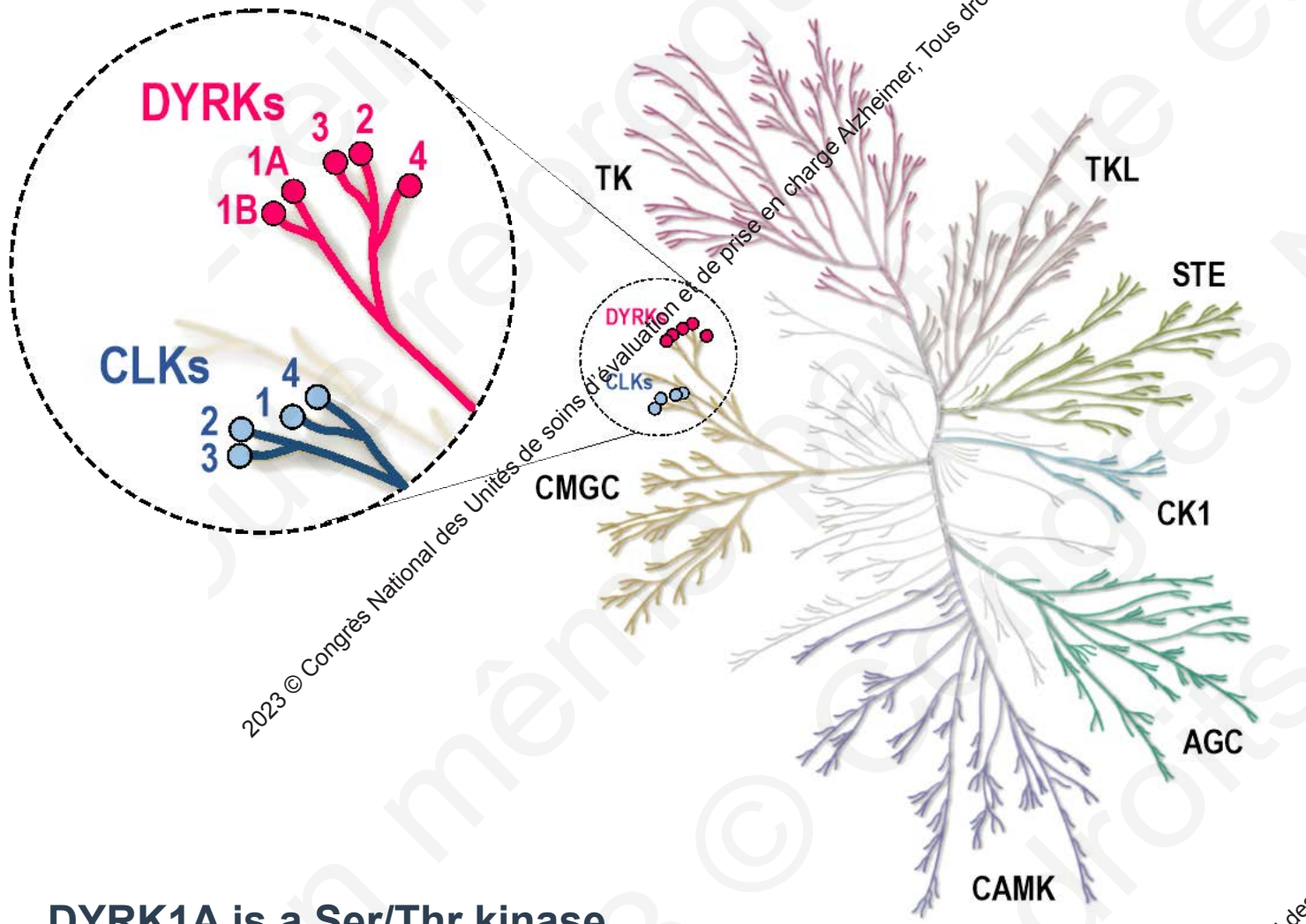
Dual specificity, tyrosine phosphorylation activated kinases (DYRKs)



DYRK1A is a Ser/Thr kinase
7 splice variants, 5 isoforms (~60-85 kDa)

- 538 kinases in the human kinome regulate protein phosphorylation
- *DYRK1A* gene located on **chromosome 21**
- Abnormal **DYRK1A kinase activity** involved in **cognitive disorders** (memory, learning) associated with **Down syndrome** (DS) & **Alzheimer's disease** (AD)
- **Inhibiting / normalizing DYRK1A corrects cognitive disorders** in animal models of DS & AD

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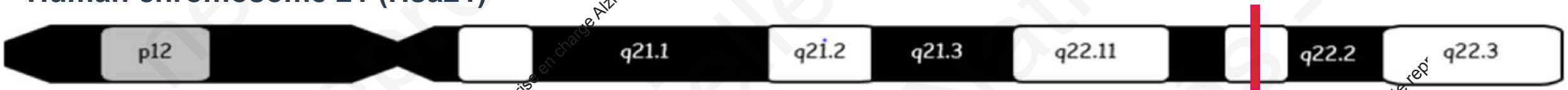
Chromosome 21, *Dyrk1a*, Down Syndrome & Cognitive Impairments

Human chromosome 21 (Hsa21)

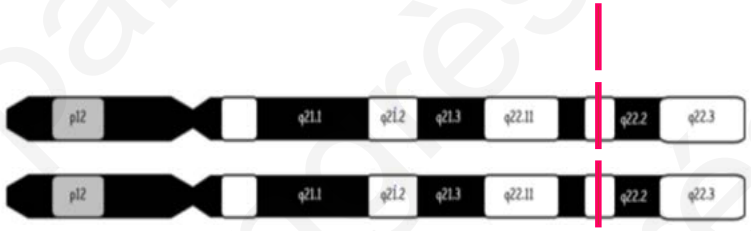


Dyrk1A (21q22.13)

A. Wild-type

NO cognitive impairments

B. Tg(*Dyrk1a*)



cognitive impairments

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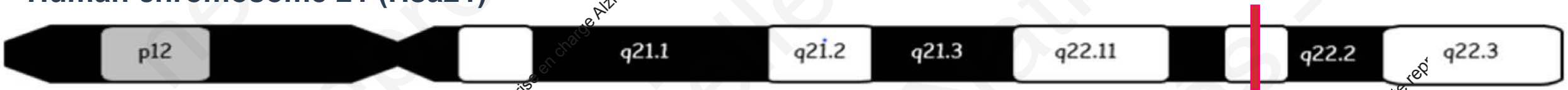

Dyrk1A (21q22.13)

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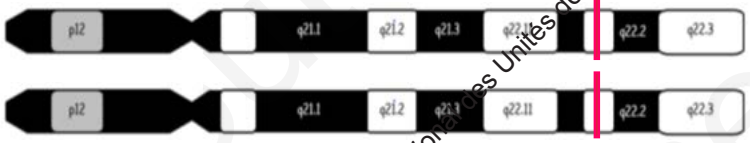
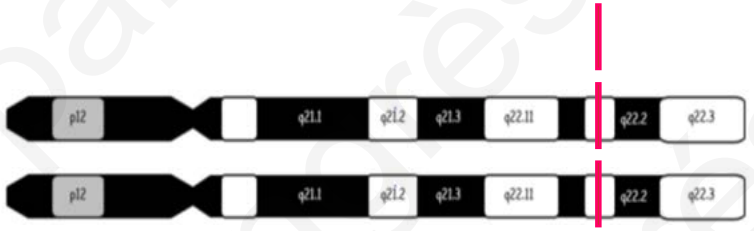


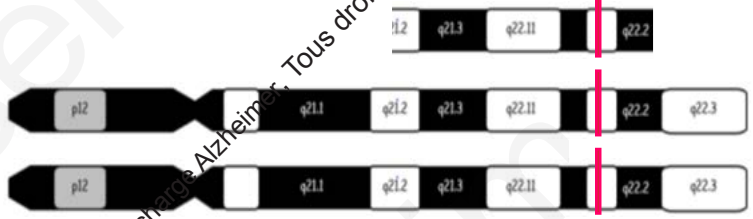
NO cognitive impairments

B. Tg(*Dyrk1a*)



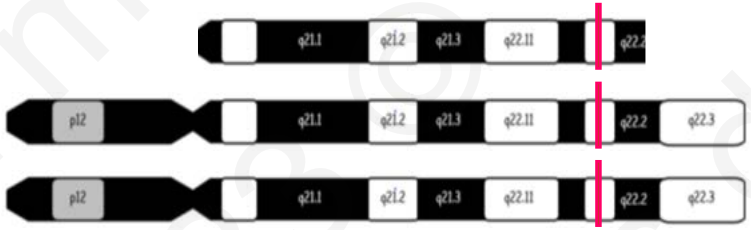
cognitive impairments

C. Ts65Dn (56.5%)



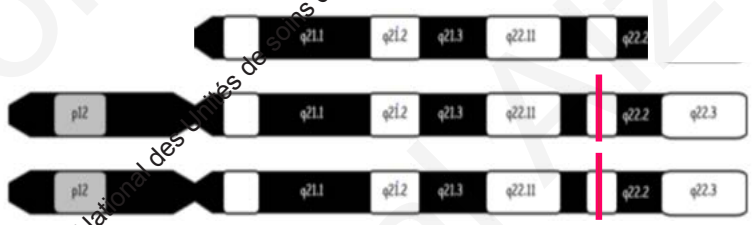
cognitive impairments

D. Dp1Tyb (63.0%)



cognitive impairments

E. Dp1Tyb (*Dyrk1a*$^{+/+/-}$)



NO cognitive impairments

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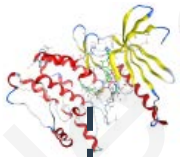
DYRK1A inhibitors as therapeutic drugs in DS and AD

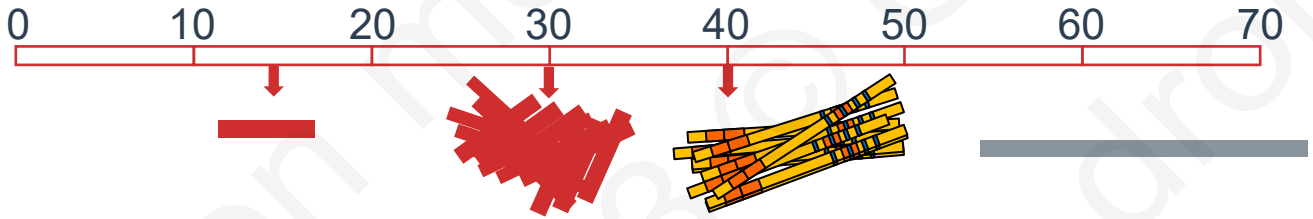
People with Down Syndrome

- Gene located in the DS critical region
- DYRK1A overexpressed and over-active in DS
- DYRK1A phosphorylates key synaptic & cytoskeleton proteins
- Excess DYRK1A dysregulates Tau mRNA splicing
- DYRK1A inhibitors correct cognitive impairments in DS animal models
- Genetic normalization of DYRK1A level corrects cognitive impairments



People with Alzheimer's Disease

- Protein and mRNA overexpressed in human AD brain
- Calpain-induced DYRK1A cleavage in human AD brain
- DYRK1A phosphorylates key regulators of AD (Tau, APP, Presenilin, Septin, Neprylysin)
- DYRK1A primes GSK-3 substrates
- DYRK1A regulates Tau mRNA splicing
- DYRK1A inhibitors correct cognitive impairments in AD animal models (3XTG-AD, APP/PS1, Aβ25-35)



APP, BACE2 & DYRK1A genes
Are located on **chromosome 21**

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From marine natural product to Leucettinib-21

50,000 compounds library screened on DYRK1A

Leucettamine B (natural product hit)

2005-2018

Leucetta microraphis (marine calcareous sponge)

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From marine natural product to Leucettinib-21

50,000 compounds library screened on DYRK1A **2005-2018**

Leucettamine B (natural product hit)

Leucettines (medicinal chemistry: +500 products synthesized)

1 patent, 2007

Leucettine L41 (Lead 1, Proof-of-Concept)

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2005-2018

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Leucettines (medicinal chemistry: +500 products synthesized)

1 patent, 2007

Leucettine L41 (Lead 1, Proof-of-Concept)

Leucettinibs (medicinal chemistry: +670 products synthesized)

2019-2023

Leucettinibs EMD-10, -92, -173 (Leads 2, PoC)

1 patent application, 2020
3 patents applications, 2021

Leucettinibs (multi-parametric selection of candidates short list)

Leucettinib-21 (clinical drug candidate)

Regulatory preclinical toxicology studies

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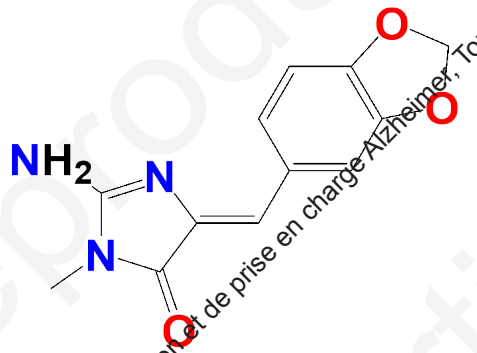
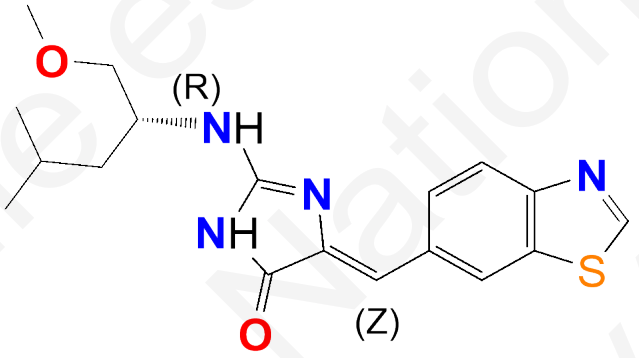
From marine natural product to Leucettinib-21

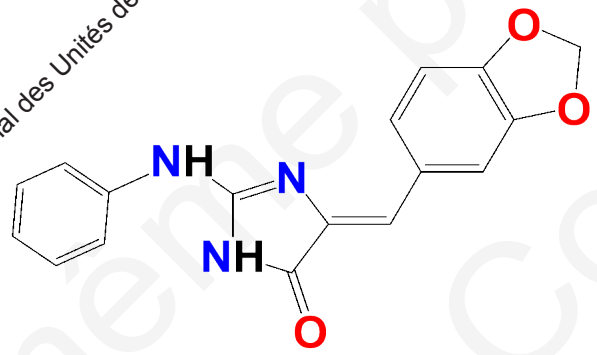


Leucettamine B

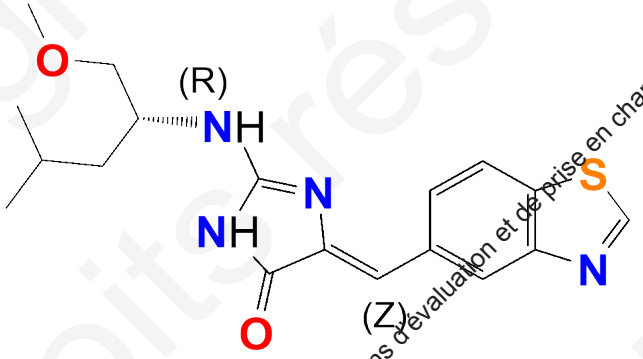


Leucettinib-21

IC_{50} (DYRK1A): 2.4 nM



Leucettine L41



iso-Leucettinib-21

IC_{50} DYRK1A: >10 µM

(**K**inase **I**nactive **I**somer)

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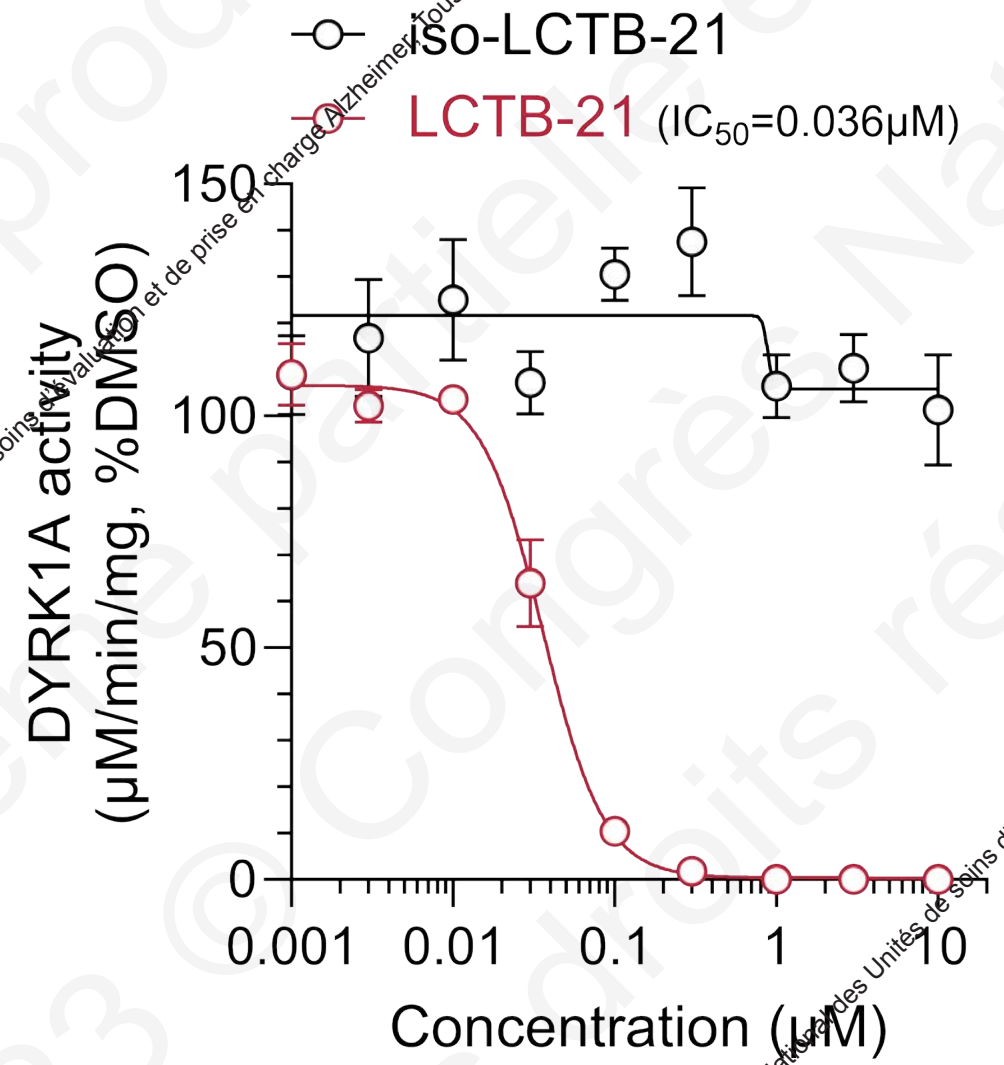
Lindberg M et al., 2023. Comparative efficacy and selectivity of pharmacological inhibitors of DYRK and CLK protein kinases. **J Med Chem 66**, 4106.
Deau E. et al, 2023. Leucettinibs, a class of DYRK/CLK kinases inhibitors inspired by the marine sponge natural product Leucettamine B. **J. Med. Chem 66**., 10694.
Lindberg et al., 2023. Chemical, biochemical, cellular and physiological characterization of Leucettinib-21, a Down syndrome and Alzheimer's disease drug candidate**. J. Med. Chem. 66**, on-line.

Leucettinib-21, not iso-Leucettinib-21, inhibits DYRK1A activity in HT-22 cells



HT-22 cells are immortalized mouse hippocampal cells

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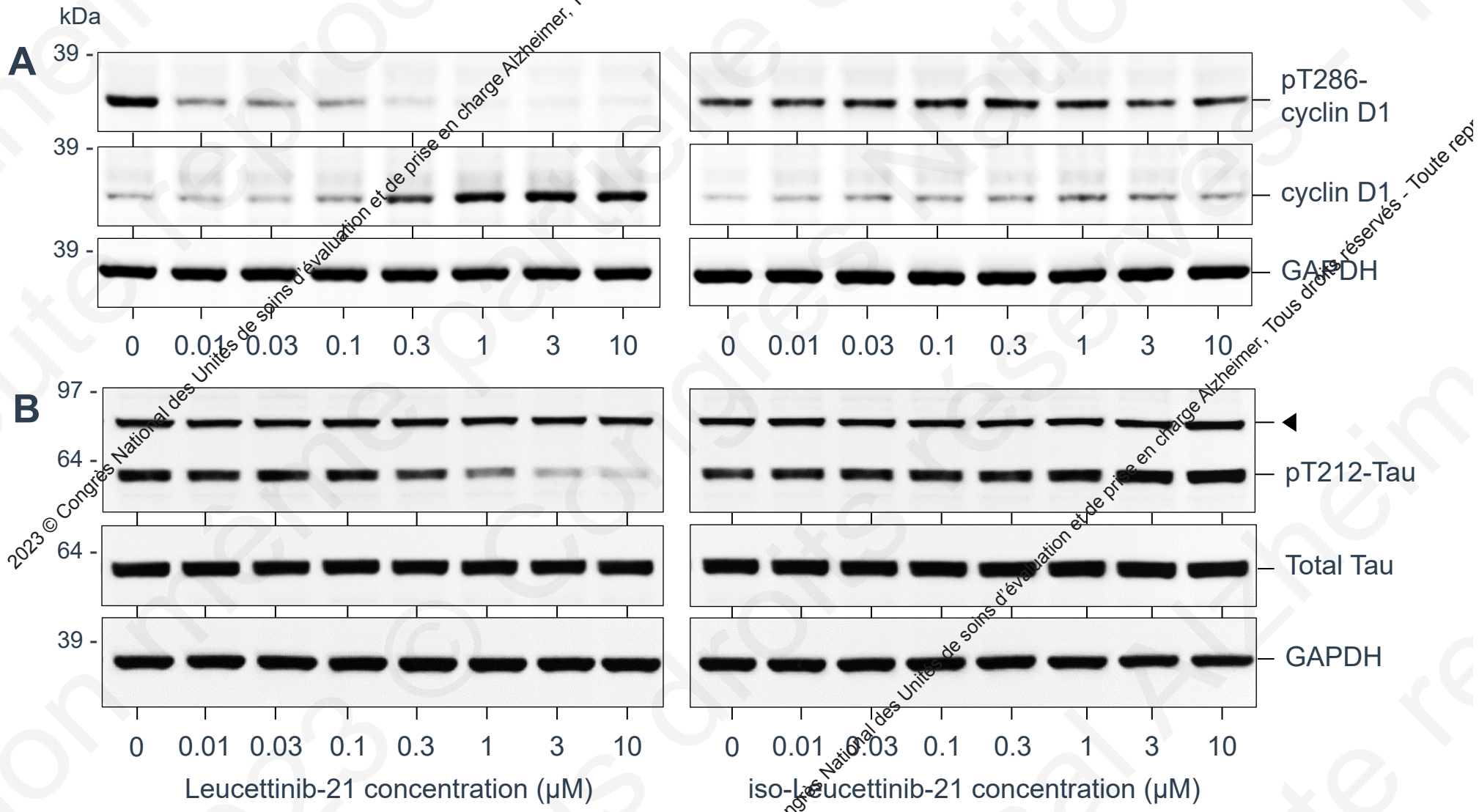
Lindberg et al., 2023. Chemical, biochemical, cellular and physiological characterization of Leucettinib-21, a Down syndrome and Alzheimer's disease drug candidate. **J. Med. Chem. 66**, in press

Leucettinib-21, but not iso-Leucettinib-21, inhibits the phosphorylation of DYRK1A substrates in SH-SY5Y cells



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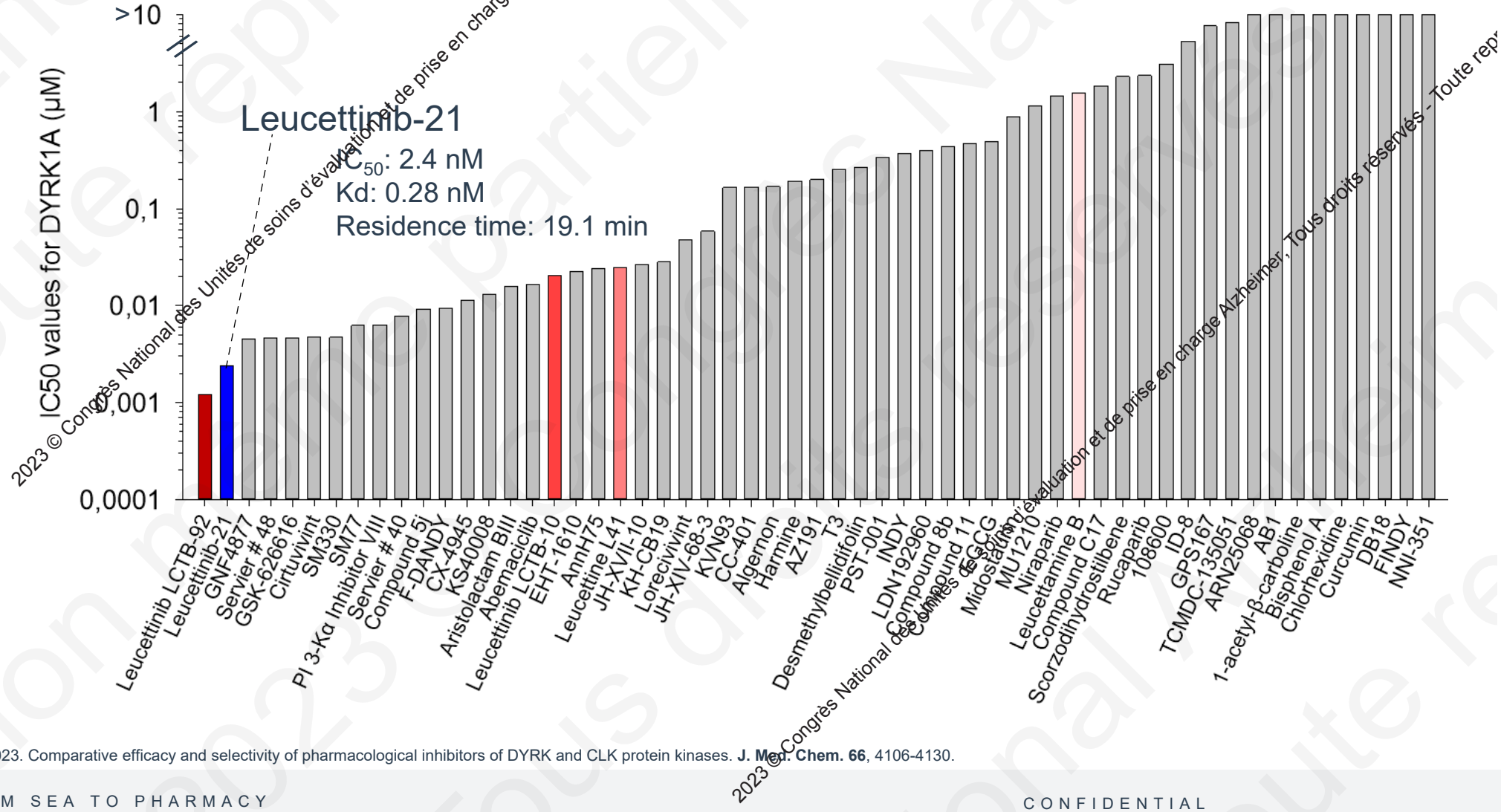
Lindberg et al., 2023. Chemical, biochemical, cellular and physiological characterization of Leucettinib-21, a Down syndrome and Alzheimer's disease drug candidate. **J. Med. Chem. 66**, in press

Benchmarking Study

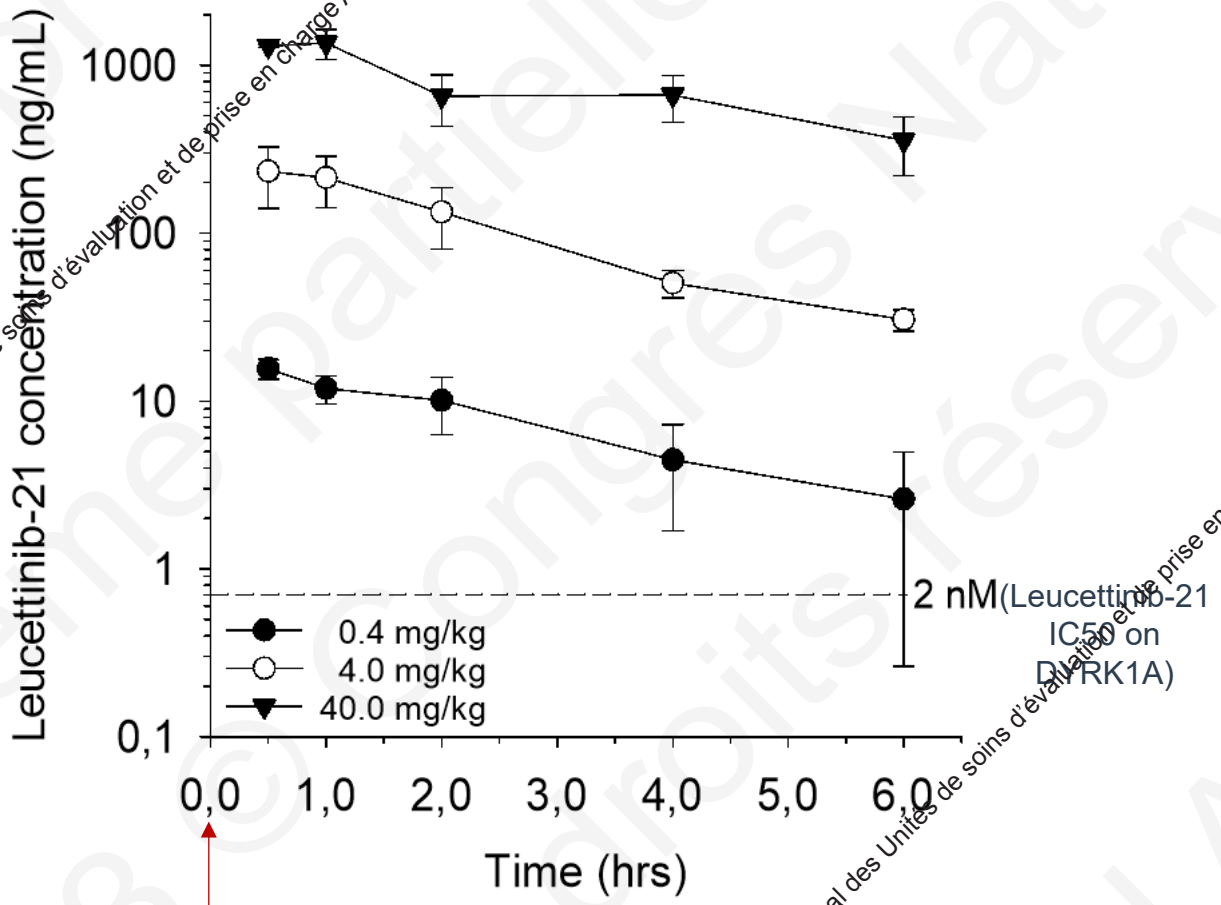
56 reported DYRKs/CLKs inhibitors tested on 12 kinases



Lindberg, M et al., 2023. Comparative efficacy and selectivity of pharmacological inhibitors of DYRK and CLK protein kinases. **J. Med. Chem. 66**, 4106-4130.

Leucettinib-21 is orally available

Pharmacokinetics in Wistar han rats



Leucettinib-21

Vehicle: Kolliphor EL / NaCl 0.9% (5/95)

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Efficacy in Down Syndrome and Alzheimer's Disease Rodent Models

Leucettines and Leucettinibs correct their cognitive impairments

1 Leucettines

Three Down Syndrome mouse models
- Tg(Dyrk1a) mice
- Ts65Dn mice
- Dp(16)1Yu mice

Two Alzheimer's disease mouse models
- ICV injected Aβ25-35 peptide
- APP/PS1ΔE9 mice

One Alzheimer's disease rat model
- AAV APP/PS1

2 Leucettinibs

Five Down Syndrome models
- Tg(Dyrk1a) mice (Y. Hérault) (LCTB-10, -92, -21)
- Dp(16)1YeY mice (Y. Hérault) (LCTB-21)
- Ts65Dn mice (M. Dierssen) (LCTB-21)
- Dp1Tyb mice (V. Tybulewicz) (LCTB-21)
- DS rat model (Y. Hérault) (LCTB-21)

Seven Alzheimer's disease models
- ICV-injected Aβ25-35 peptide in mice (LCTB-10, -92, -21) (Amylgen)
- 5x-FAD, Denali APP-KI models (C. Lemere)
- Tau model (Remynd)
- Aging rat (Portsolt)
- Streptozotocin ICV-injected rat (Portsolt)
- Goto-Kakizaki T2D rat (J. Movassat)
- HSV-1 infected mice (G. De Chiara)

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Naert G et al., 2015. Leucettine L41, a DYRK1A-preferential DYRKs/CLKs inhibitor, prevents memory impairments and neurotoxicity induced by oligomeric Aβ25–35 peptide administration in mice. European Neuropsychopharmacology 25, 2170–82.
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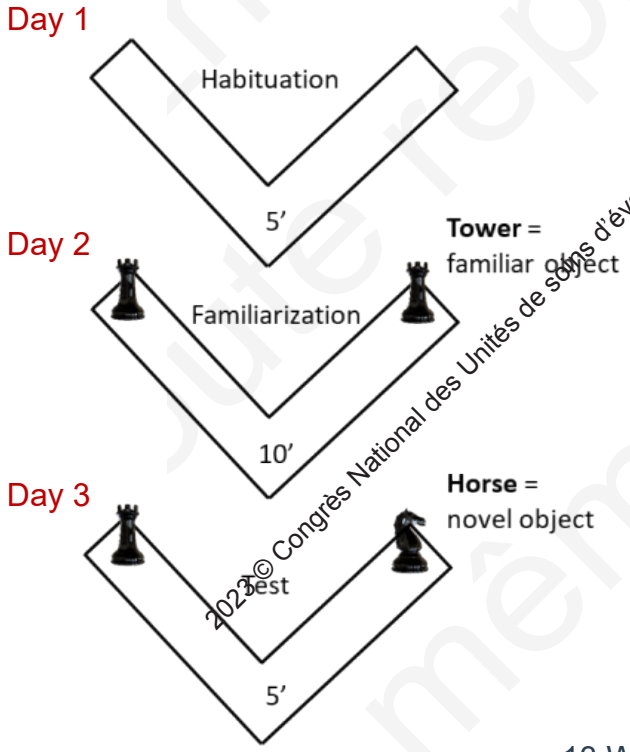
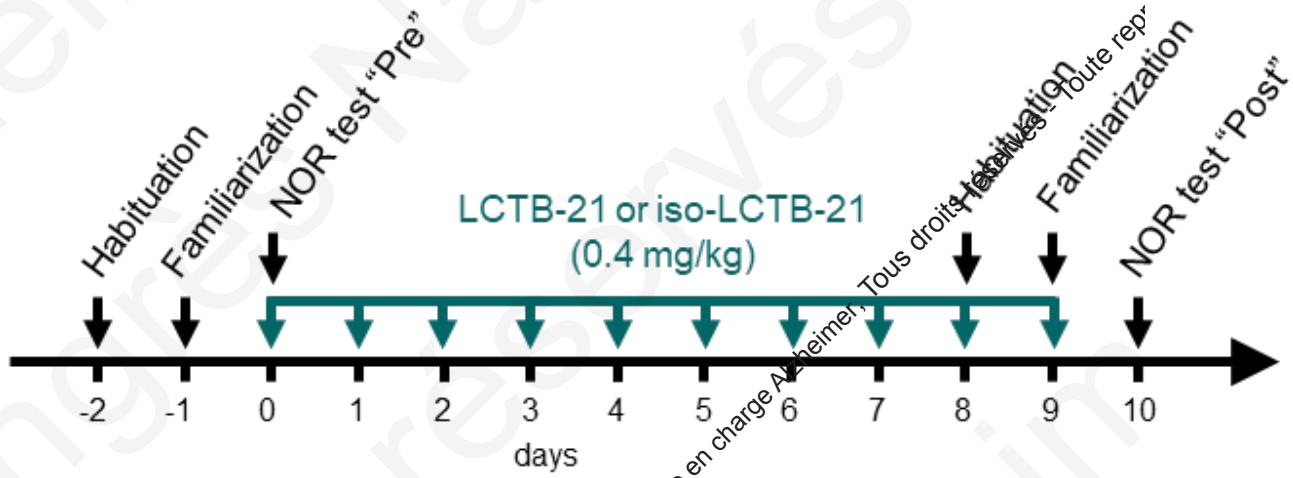
Leucettinib-21 corrects cognitive impairments in Ts65Dn mouse model

Oral gavage administration (0.4 mg/kg daily for 10 days) rescues memory impairment in Ts65Dn mice (Novel Object Recognition test)





13 Wild-type (WT)

13 Ts65Dn

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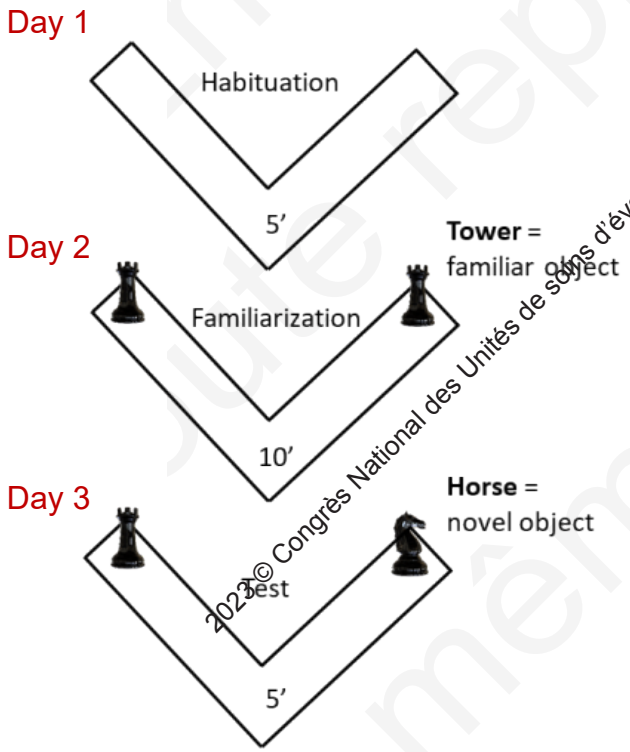

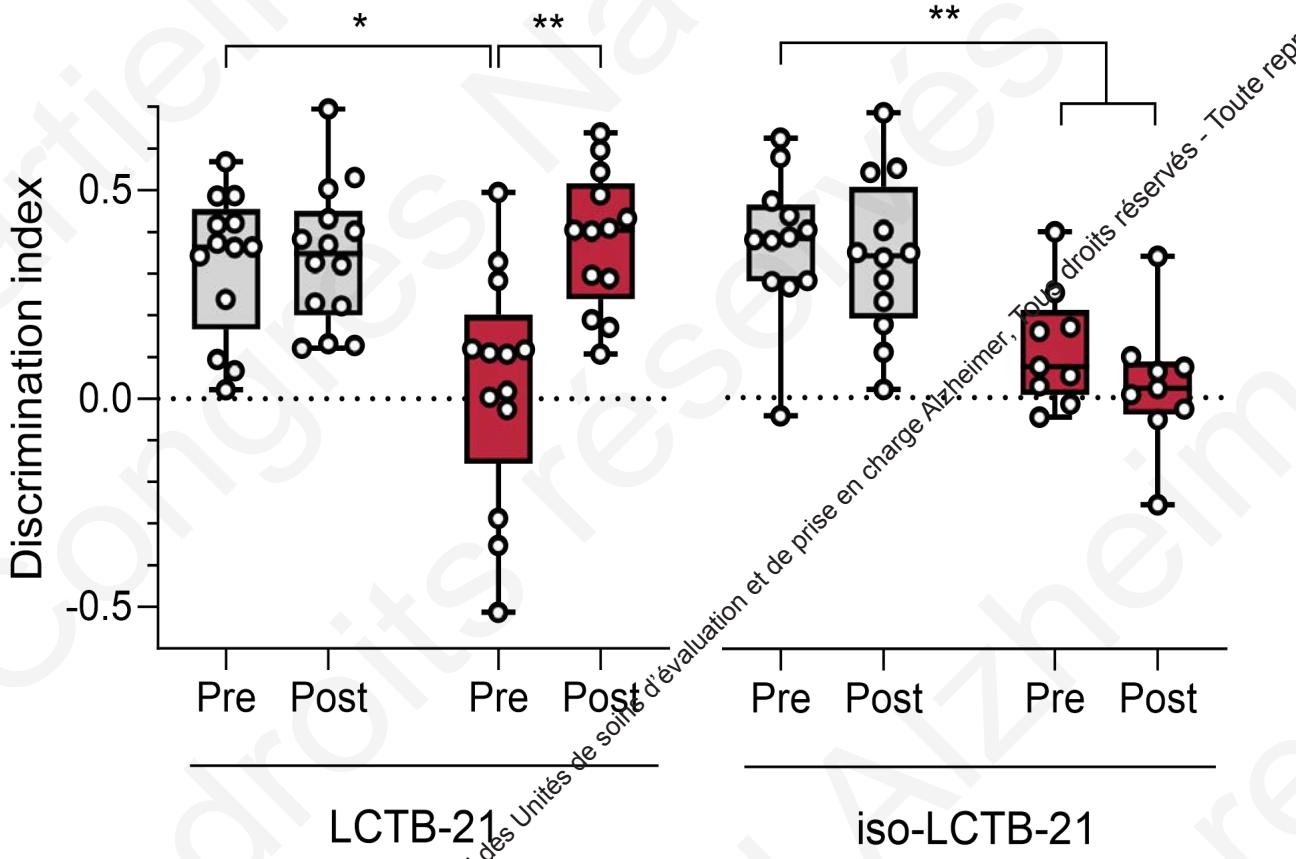

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Alvaro FERNANDEZ, Mara DIERSSEN, Barcelona

Lindberg et al., 2023. Chemical, biochemical, cellular and physiological characterization of Leucettinib-21, a Down syndrome and Alzheimer's disease drug candidate. **J. Med. Chem. 66**, on-line

Molecular mechanisms of action of Leucettinibs

Relevant biomarkers for Down Syndrome and Alzheimer's disease

DYRK1A →

Protein substrate	Site
Amphiphysin (AMPH)	Ser-293
Amyloid Precusor Protein (APP)	Thr-668
Cyclin D1 (CCND1)	Thr-286
Cyclin D2 (CCND2)	Thr-280
Cyclin D3 (CCND3)	Thr-283
Dynamin 1 (DYN1)	Ser-857
Forkhead box protein O1 (FOXO1)	Ser-329 (326 in mouse)
Glycogen synthase kinase 3 (GSK3)	Thr-356
Munc18-1	Thr-479
Myocyte Enhancer Factor 2D (MEF2D)	Ser-251
Neprilysin (NEP)	direct or indirect?
N-methyl-D-aspartate glutamate receptor 2A (GluN2A)	Ser-1048
Nuclear distribution element-like 1 (NDEL1)	DYRK2: Ser-336
Nuclear factor of activated T cells (NFATc4)	Ser-215
p27Kip1 (CDKN1B)	Ser-10
Presenilin 1 (PS1)	Thr-354
Regulator of Calcineurin 1 (RCAN1)	Ser-112, Thr-192
Septin 4 (SEPT4)	direct or indirect?
Signal transducer and activator of transcription 3 (STAT3)	Ser-727
Synapsin (SYN)	Ser-551
Synaptojanin 1 (SYNJ1)	Ser-1029 (*Drosophila*)
α-Synuclein (SNCA)	Ser-87
Tau	Thr-212

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Molecular mechanisms of action of Leucettinibs

Down syndrome: X 1.5 expression & activity of DYRK1A
Alzheimer's disease: calpain-induced LMW-DYRK1A, more active & more stable

1. Abnormally increased DYRK1A kinase activity observed in DS (*Dyrk1a* gene on chromosome 21) & AD (activation by proteolytic cleavage) leads to cognitive impairments (spatial & learning memory)

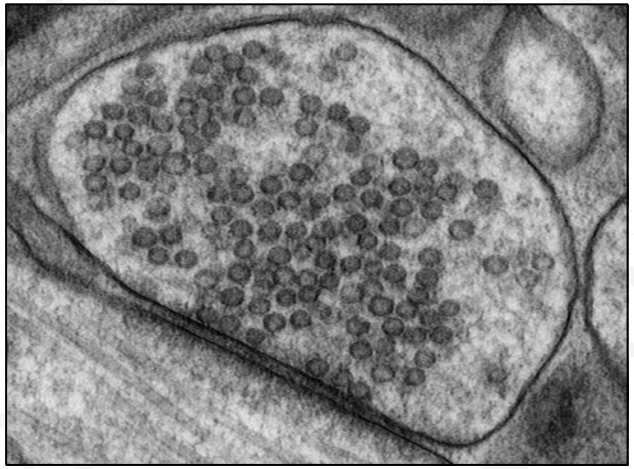
2. Inhibiting DYRK1A's excess activity corrects cognitive impairments (memory, learning) associated with Down Syndrome & Alzheimer's disease

3. Chromosome 21 contains the *Dyrk1a* gene as well as the genes of *Amyloid Precursor Protein* (*APP*) and *BACE2* (*APP-Cleaving Enzyme*), 3 key players in the development of AD. People with DS have a very high risk to develop early-onset AD, and all develop dementia later

DYRK1A

Leucettinib-21

Synapses



Phosphorylation of pre-/post-synaptic proteins

Synaptic plasticity, vesicle dynamics, stability, turnover

Neuronal connectivity

Sources: Arbones ML et al., 2019. DYRK1A and cognition: A lifelong relationship. **Pharmacol Ther 194**, 199. / Laham AJ et al., 2021. DYRK1A: a down syndrome-related dual protein kinase with a versatile role in tumorigenesis. **Cell Mol Life Sci 78**, 603. / Atas-Ozcan H et al., 2021. Dyrk1a from gene function in development and physiology to dosage correction across life span in Down Syndrome. **Genes 12**, 1833. / Lindberg MF & Meijer L, 2021. Dual-specificity, tyrosine phosphorylation-regulated Kinases (Dyrks) and Cdc2-like Kinases (Clks) in human disease, an overview. **Int J Mol Sci 22**, 6047. / Deboever E et al., 2022. The omnipresence of DYRK1A in human diseases. **Int J Mol Sci 23**, 9355.

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Leucettinib-21 displays a Wide Safety Margin

Preliminary toxicity MTD/DRF		GLP 4-week toxicity with recovery	
Oral administration	Oral administration	Oral administration	Oral administration
14 days	21 days	28 days	28 days
40, 80,140 mg/kg	60 mg/kg	45, 90, 180 mg/kg	10, 20 mg/kg
6 animals / group	6 animals / group	20 animals / group	20 animals / group

Preliminary toxicity MTD/DRF	GLP 4-week toxicity with recovery
Oral administration	Oral administration
10 days	28 days
30, 100 mg/kg	20, 40,100 mg/kg
4 animals / group	6 - 8 animals / group

- No mortality, severe toxicity or deterioration of clinical conditions were apparent following repeated oral administrations of Leucettinib-21 at doses up to 45 mg/kg in rats, and 100 mg/kg in minipigs.
- The main toxic effects were haematological changes with a decrease in lymphocytes and in red blood cells and histopathological lesions in several organs. These effects were dose dependent.
- The **NOAELs** were established as **20 and 100 mg/kg in rats and minipigs**, respectively (well above the pharmacologically active doses in rodents at 0.3 to 0.5 mg/kg) suggesting a significant safety margin.

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Leucettinib-21

Immediate release minitablets are adapted to a pediatric population since they combine the inherent advantages of oral solid dosage form and the possibility of personalized dosing.

Overview of CMC data

GMP Drug Substance

- Completed production of 1 kg GMP drug substance batch with
- On-going 36-month stability study
- 99.8% purity

GMP Drug Product

- 60 mg immediate release minitablets containing 3.5 mg of drug substance
- Completed production of around 20,000 GMP tablets, including 3,000 placebo tablets
- 18 months expiration support
- On-going 24-month stability study

Phase 1 Clinical Trial Materials

- Release of tablet vials to the phase 1 clinical center expected in December 2023

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Phase 1 clinical study (Leucetta)

(Yves DONAZZOLO, Eurofins - Optimed, Grenoble)

Double-blind, placebo-controled safety trial

PK and PD data, plasma proteomics & phosphoproteomics

Leucettinib-21

Single Ascending Dose (**SAD**) (single dose)

*(5, 10, 20, 20 bid, 40, 60 mg - **48** participants: 6 LCTB-21 + 2 placebo /dose)*

Food Effect (**FE**) (single dose)

*(20 mg – **12** participants: 6 fed/ 6 fasted – cross-over)*

Multiple Ascending Dose (**MAD**) (daily for 14 days)

*(20, 20 bid, 40 mg - **36** participants: 9 LCTB-21 + 3 placebo /dose)*

Single Dose (**Adult DS patients**)

*(20 mg LCTB-21 - **12** participants)*

Single Dose (**AD MCI patients**)

*(20 mg LCTB-21 - **12** participants)*

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Baptismal fonts of the Laon Cathedral, France (12th century)

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from Sea to Pharmacy

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