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ADeBIOTeCH

THINK TANK ONE HEALTH

Reducing animal use in efficacy studies of complex neurological diseases: good and bad news

Alternative to animal experimentation – Nicolas Violle

6th of June 2023 - Biocitech

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ETAP-Lab,
Valuable solutions for preclinical studies

European leader in preclinical Dermatology

- Psoriasis
- Atopic dermatitis
- Wound healing
- Skin grafting
- …

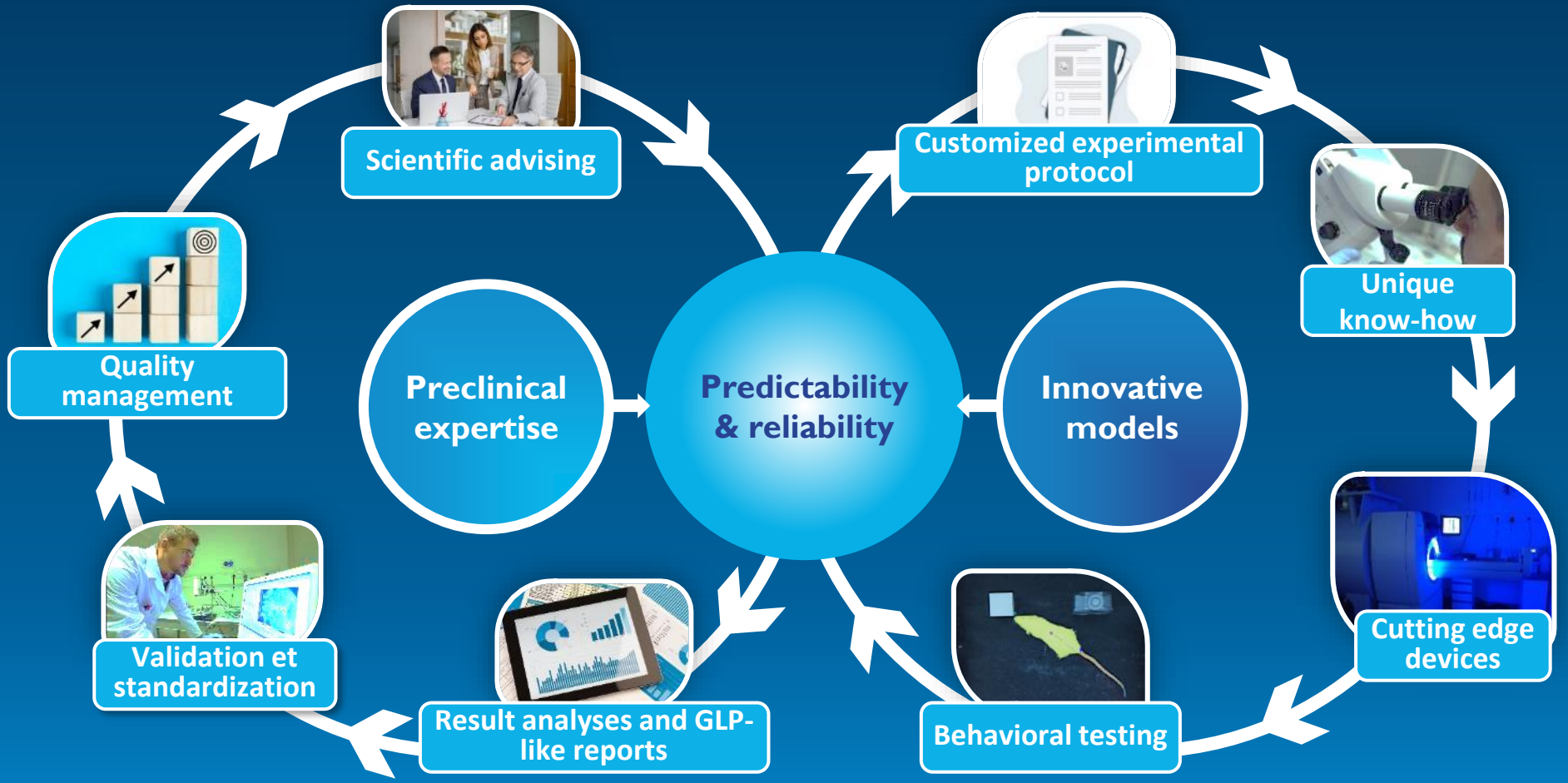
STROK@LLIANCE
The only CRO 100% dedicated to preclinical stroke

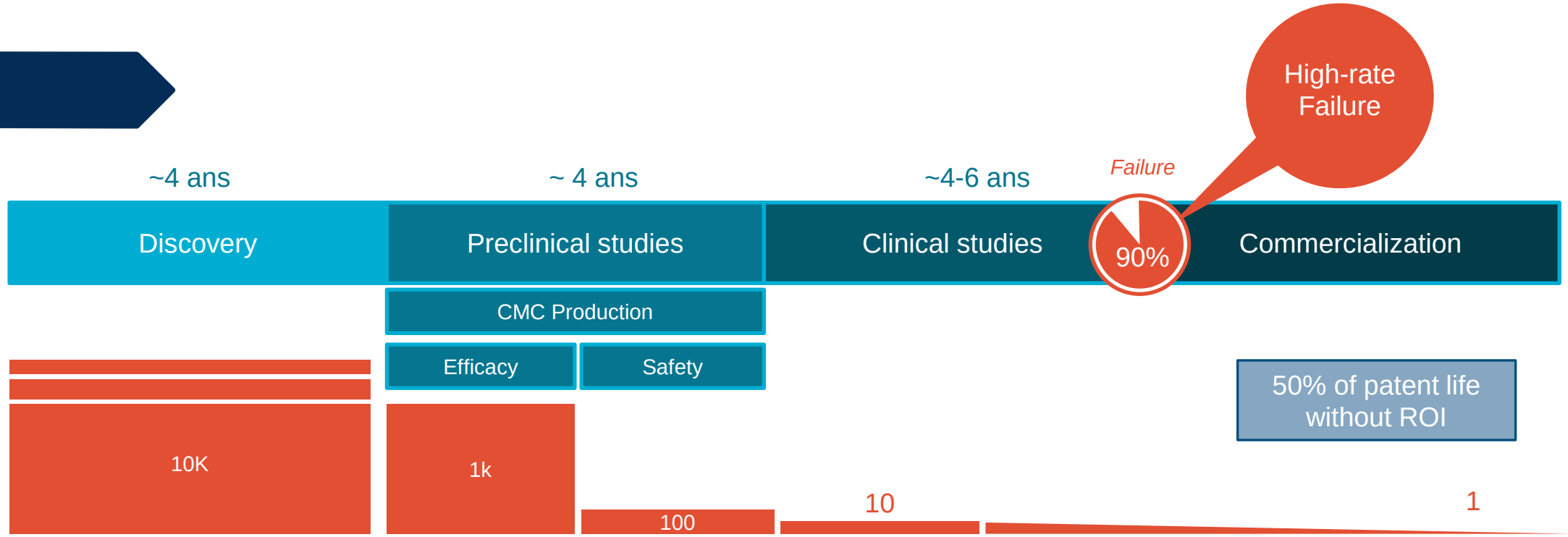
Neurodegenerative diseases
In vitro and *in vivo* models of AD and PD based on toxic soluble oligomers ($A\beta_{1-42}$, tau, α-syn)

Pioneer in the field

- Gut-brain axis
- Gut inflammation
- Functional food
- Food toxicology
- …

A 360° know-how for best-in-class services





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In vivo and In vitro test to select the best effective drugs

High-quality selection to reduce time-to-market, save lifes and money

1-2 billion €
cost/drug on market

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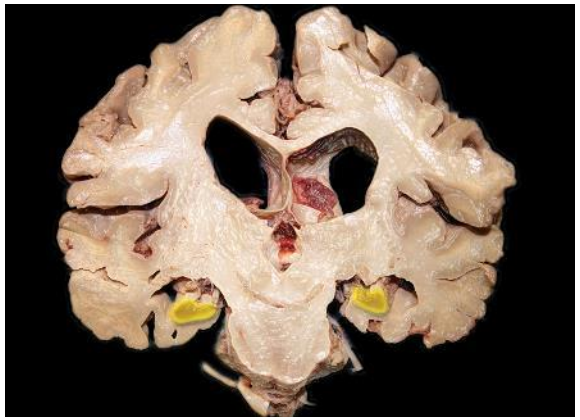
Pharmacology of neurological disease : bridging the translational gap?

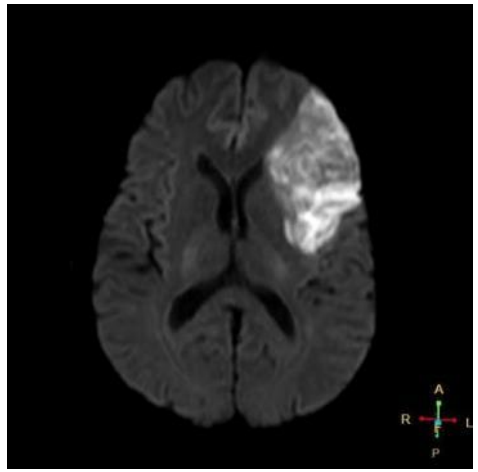
Alzheimer's disease

- 75% of neurodegenerative disease cases
- Sporadic form 94%
- 50 million peoples worldwide in 2020
- ≈100 drug failures in clinic phase II-III last 10 years
- 2% success rate (under debate)



Cerebral stroke

- 2nd cause of death worldwide
- 13.7 million cases in 2016
- >1000 molecules reported with protective effects
- >250 clinical trials performed
- 1 drug



CAN WE DO BETTER?

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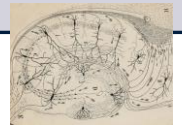
Alzheimer's disease

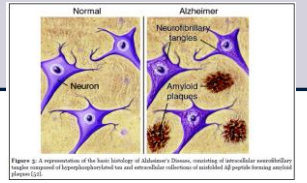
1790's
Luigi Galvani
Animal electricity
(Frog)

1848-1849
Du Bois-Raymond & Helmotz
Action potential
(Frog)

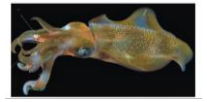
1888
Ramon y Cajal
Neuron doctrine
(Nobel prize 1906)
(Bird)



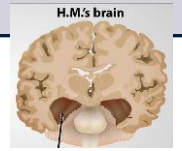
1901
Alois Alzheimer
Pathology first description
(Human)



1952
Huxley & Hodgkin
Action potential mechanisms
(Nobel prize 1963)
(Squid)

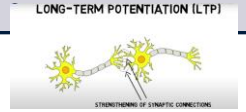


1957
Milner
Role of brain hippocampus in learning
(Human)



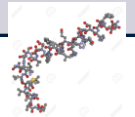
1960
Kandel
Acute vs long-term memory mechanisms
(Nobel prize 2000)
(Aplysia)

1966
Lomo & Bliss
Long-term potentiation in hippocampus
(Rabbit)

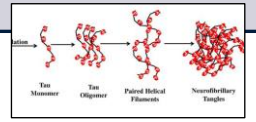


1976
Watkins
NMDA receptor
(Cat & Frog)

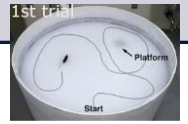
1984
Glenner & Wong
Amyloid beta peptide identification
(Human)



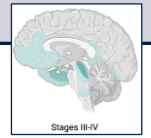
1986
Kosik et al.
Tau identification in tangles
(Human)



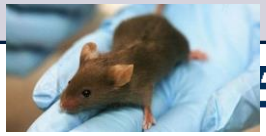
1986
Morris
Role of NMDA and LTP in hippocampus for spatial memory *(Rat)*



1990's
Braak & Braak
Spatiotemporal distribution of tau
(Human)



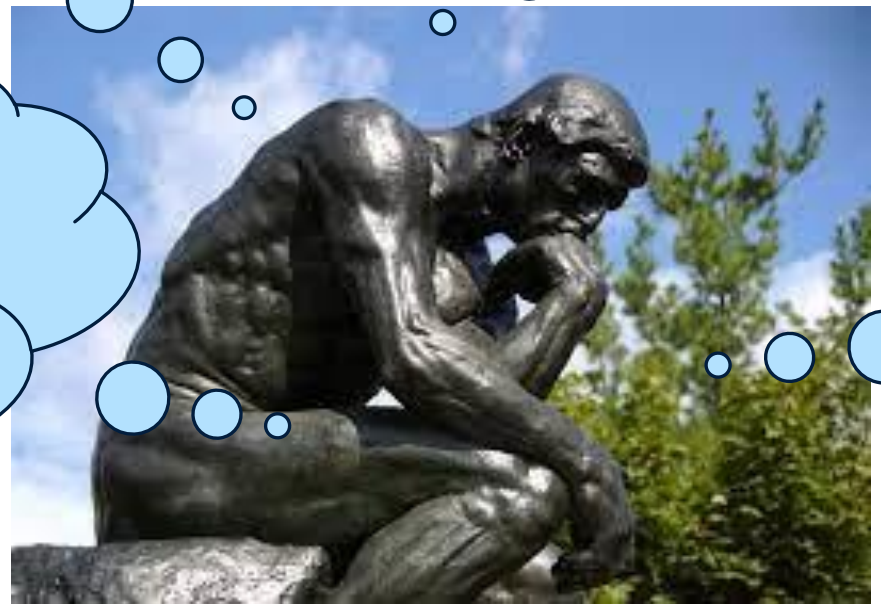
1995
Games et al.
First transgenic model of AD (Aβ hypothesis)
(Mouse)



Alzheimer's disease

Why animal models are relevant to unravel CNS biology but unpredictive for CNS deseases?

How can we model a pathology that we do not really understand?



Can we do better with NAMs?

Is using human cells enought and sufficient to improve?

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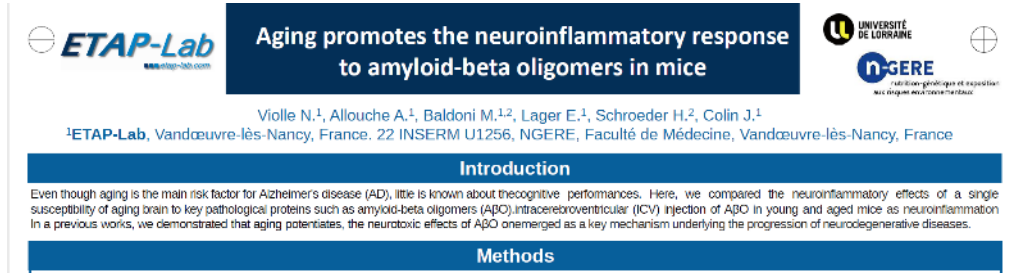
Alzheimer's disease

Two strategies at ETAP-Lab:

1. **Refining our animal models**
   - ✓ Wild-type models
   - ✓ Adding risk factors (i.e. age, sex,…)
   - ✓ Respecting chronobiology of the animals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Aging promotes the neuroinflammatory response to amyloid-beta oligomers in mice

UNIVERSITÉ DE LORRAINE

nGERE

Violle N.[1], Allouche A.[1], Baldoni M.[1,2], Lager E.[1], Schroeder H.[2], Colin J.[1]

[1]ETAP-Lab, Vandœuvre-lès-Nancy, France. 22 INSERM U1256, NGERE, Faculté de Médecine, Vandœuvre-lès-Nancy, France

Introduction

Even though aging is the main risk factor for Alzheimer's disease (AD), little is known about thecognitive performances. Here, we compared the neuroinflammatory effects of a single susceptibility of aging brain to key pathological proteins such as amyloid-beta oligomers (AβO).Intracerebroventricular (ICV) injection of AβO in young and aged mice as neuroinflammation In a previous works, we demonstrated that aging potentiates, the neurotoxic effects of AβO onemerged as a key mechanism underlying the progression of neurodegenerative diseases.

Methods

2. **Complexifying our cellular models**
   - ✓ iPSCs with mixed cell types
   - ✓ Using microfluidic systems to model brain connectivity

BIO-DIAMOND

Bio-Diamond project

- **Two partners**
  - **NETRI** => microfluidic devices to create brain-on-chips models
  - **ETAP-Lab** => Alzheimer modeling and drug testing
- The goal is to develop new innovative *in vitro* models of Alzheimer, Parkinson and ALS diseases
- **5 millions €** over 3 years, started in March 2023
- Granted by France 2030

BIO-DIAMOND

NETRI
PROVIDING ORGANS-ON-CHIP FOR THE INDUSTRY

FRANCE 2030

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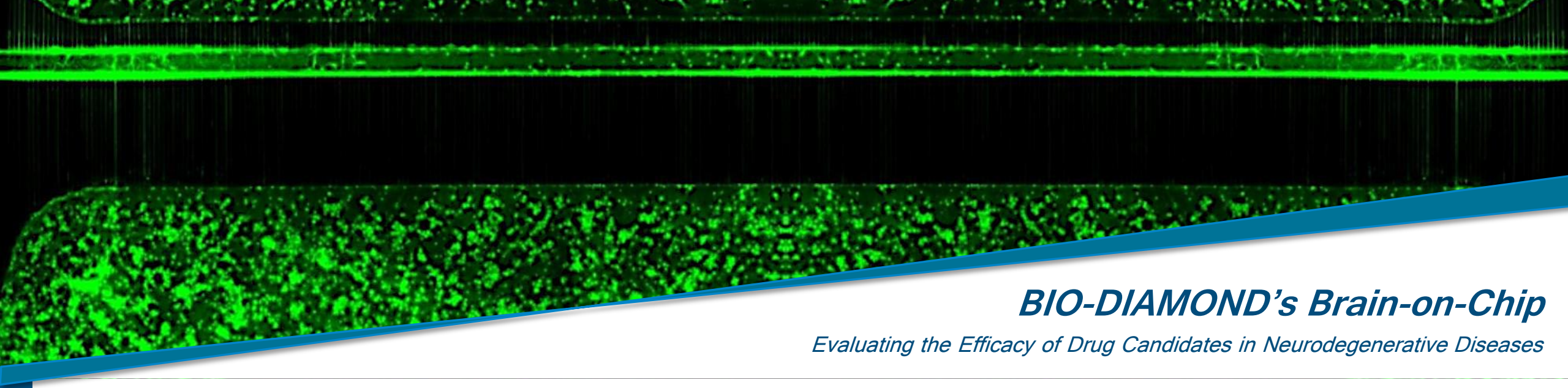


BIO-DIAMOND's Brain-on-Chip

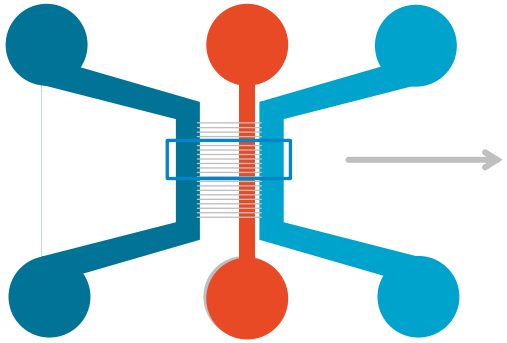
Evaluating the Efficacy of Drug Candidates in Neurodegenerative Diseases

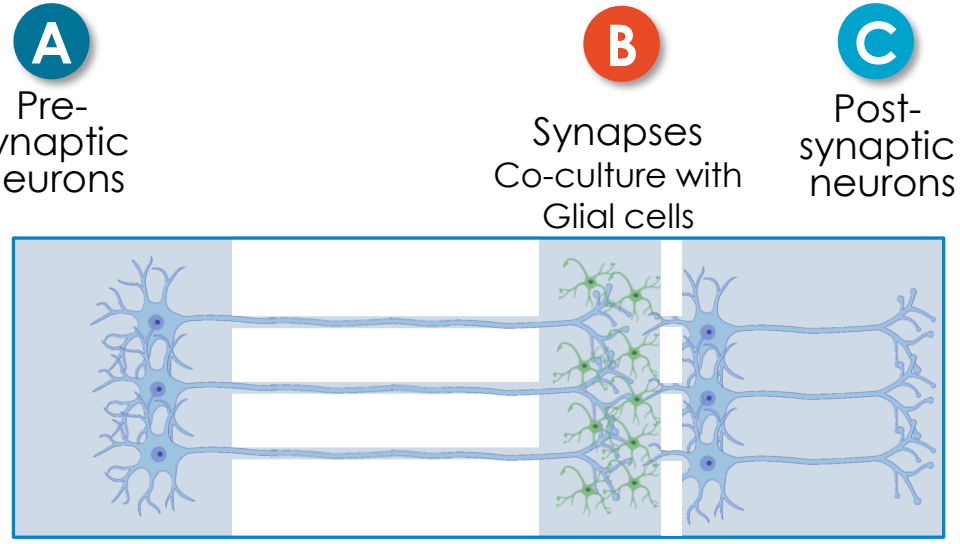
Applications

- Assessment of drug efficacy against neurodegeneration or aggregation / spreading of disease-associated proteins (Aβ, Tau, α-Syn).
- Independent access and manipulation pre- and post-synaptic compartments + synaptic region.

Assay principle

Design of the BoC





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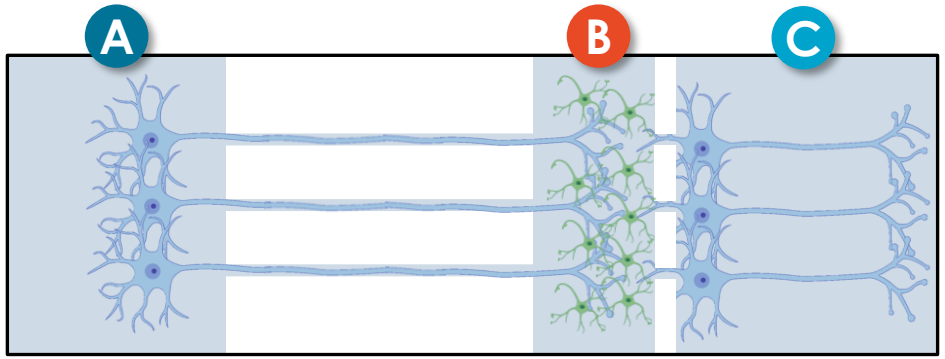
Spreading assay

Goal

Evaluating neurons-to-neurons **spreading** of Tau using microfluidically isolated chambers.

Induction

TauO



Model

- **Human** IPSCs including
  - **AD's** : Glu/GABA neurons (90/10% ratio)
- Tagged TauO in chamber **A** or **C**

Readouts

- Tagged oligomer trafficking
- Pathological phosphorylations
- Live staining
- Colocalization, internalization
- Aggregates

Tool

- High Content Screening and Analysis System with incubator for live staining (Time-lapse)

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Neurodegeneration assay

Goal

Assessing the drug efficacy on neurodegeneration in BoC models of AD's induced by amyloid beta oligomers (AβO) and/or Tau oligomers (TauO).

Model

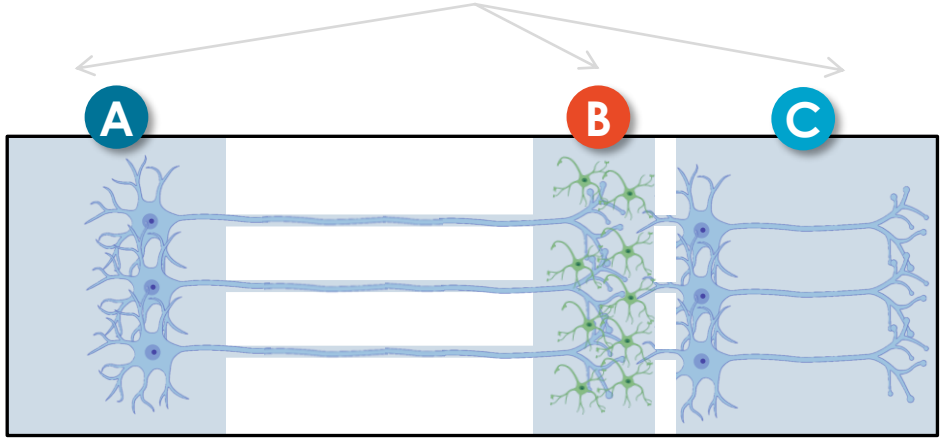
- **human IPSCs** (Glu/GABA neurons in a 90/10% ratio)
- AβO and/or TauO in chamber **A**, **B** or **C**

Readouts

- Synaptic & vesicular markers
- Autophagy, endocytosis, phagocytosis (microglia)
- Axonal & soma degeneration
- Morphological analysis
  - Dendritic spines
  - Neurite length
  - Network density
- Electrophysiology

Induction

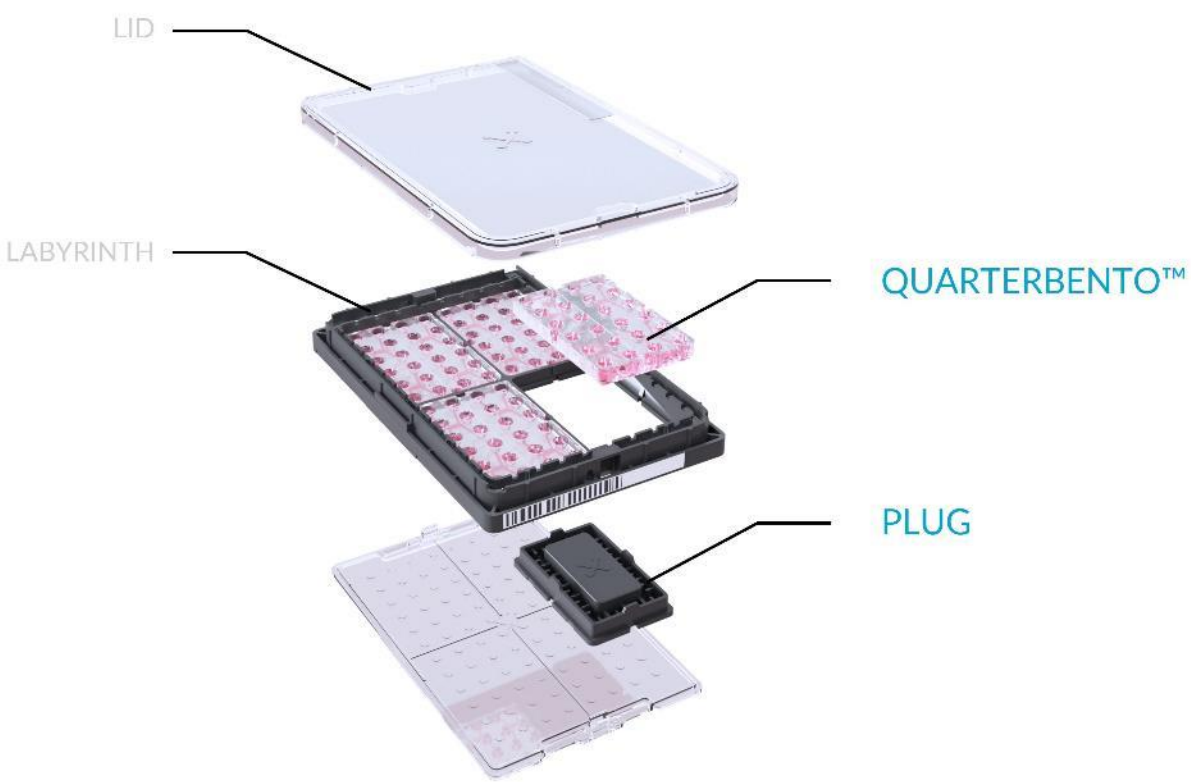
AβO and/or TauO



Tools

- Simple Plex assays (4 targets)
- High Content Screening and Analysis System
- Multielectrode array

Medium speed throughput



- ✓ 16 BoC per plate allowing replicates and multiple treatment comparisons
- ✓ Fast imaging process in living cells with Operetta CLS
- ✓ Results in 4-8 weeks after cell culture start
- ✓ Compartmentalization for more translational assays
- ✓ Need less chemicals and drugs than conventional in vitro assays

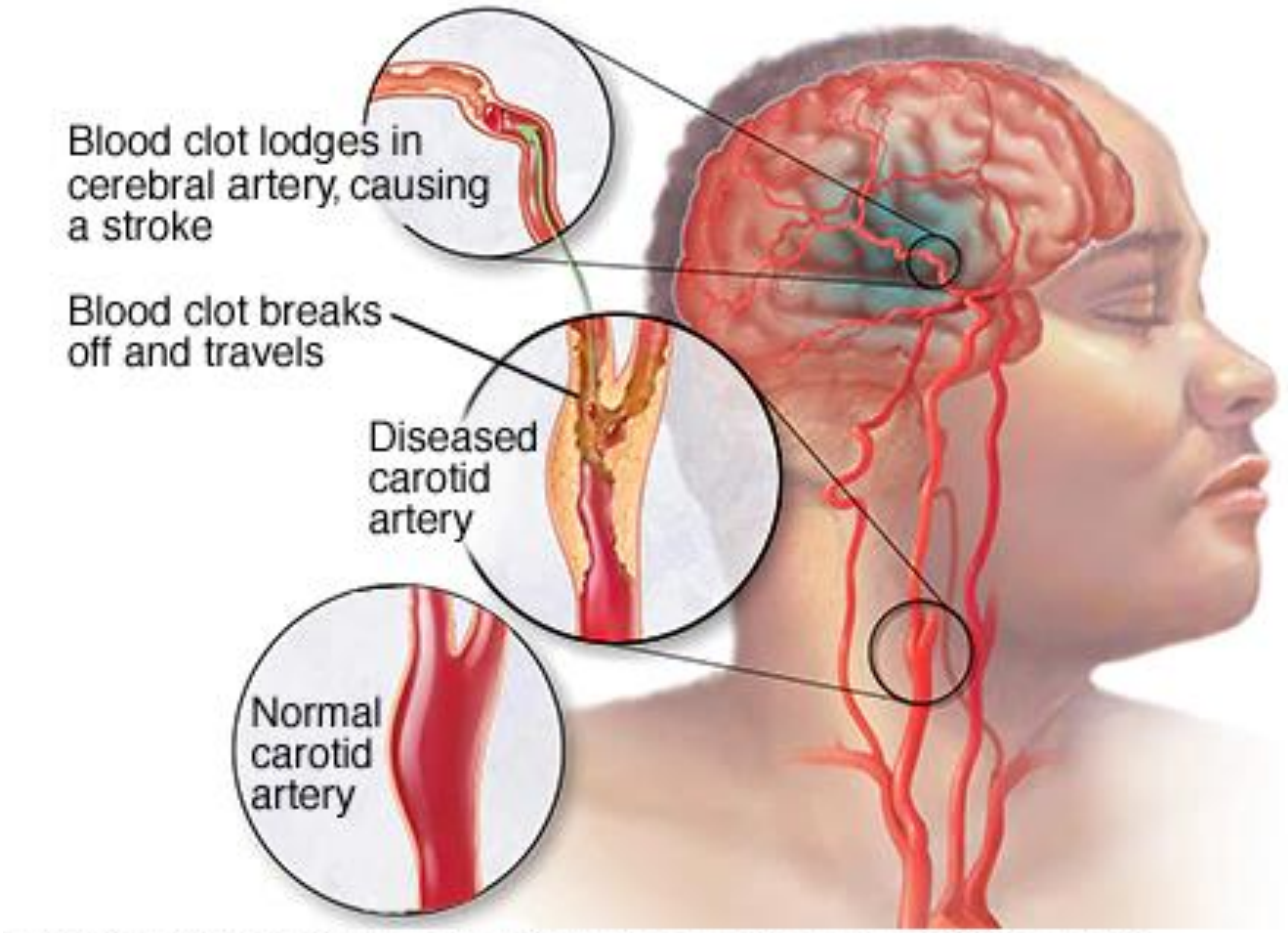
Alzheimer's disease

- **What is expected from these models?**
  - Support the understanding of the pathology
  - **<u>Expected better transnationality due to human cells + compartmentalization</u>**
  - Prerequisite to *in vivo* models for drug selection, reducing the number of animal studies
  - **<u>Excellent models for mechanistic studies</u>**

- **Potential pitfalls:**
  - The situation remain simplified (no BBB, no vessels, no CSF, no microglia, no myelin, growth factors, oxygenation, young cells in a stressing environment…)
  - How to validated cell phenotype compared to the human situation?
  - Pharmacological reference and comparators? Human variability?
  - Pathological concepts behind the models (amyloid hypothesis?)

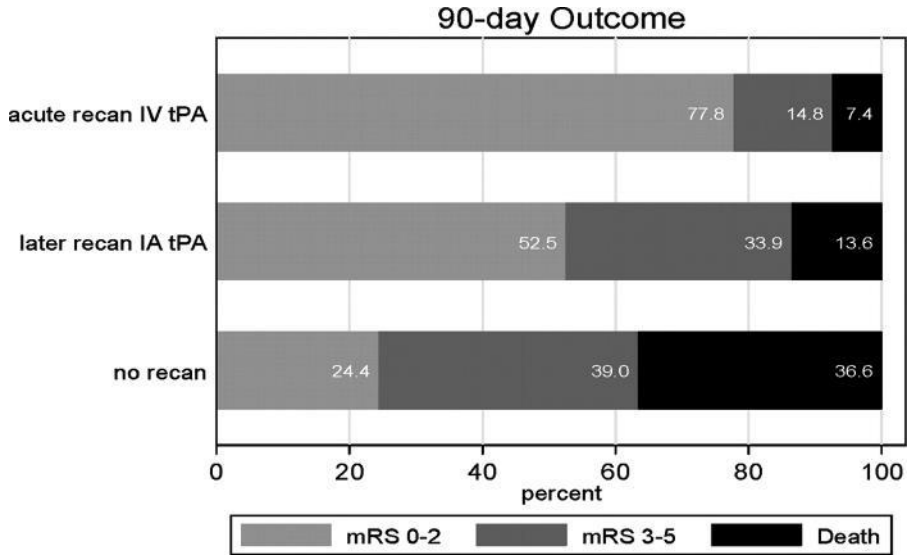
- **What will still be done in animals (even if successful)?**
  - PK/PD studies in a complete living system
  - Behavioral studies
  - Research of new mechanisms and biomarkers

Stroke models



Current treatments in stroke

- ❑ **The only effective strategy : early recanalization**
- ❑ **Two therapeutic options:**
  - ✓ **IV rt-PA (< 4.5h after stroke onset)**
    - *25% of patients treated*
  - ✓ **Thrombectomy (<6h after stroke onset)**
    - *<10% of patients treated*



From Bathia *et al.*. Stroke 2010

rt-PA (Alteplase, Actilyse) is the only approved drug for stroke

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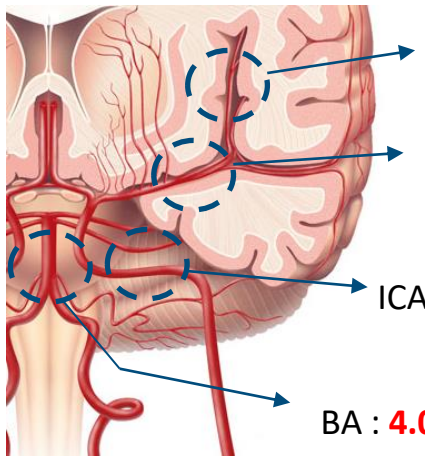
rt-PA – the only drugs for acute stroke

- **Dose of 0.9 mg/kg (10% bolus; 90% perfusion over 1h) if <4.5h after stroke onset and DWI/PWI mismatch**
- **Full recanalization rate after i.v. thrombolysis: 21.3 %**

✓ **Recanalization rate depends on occlusion site**



MCA-M2 : **30.4 %**

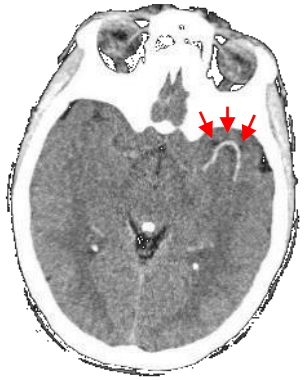
MCA-M1 : **32.8 %**

ICA : **4.4 %**

BA : **4.0 %**

From Bathia *et al.*, Stroke 2010

✓ **Recanalization rate depends on clot composition and length**



Low HU
Platelet-rich : → **5.9 %**

High HU
Erythrocyte and fibrin-rich : → **29.4 %**

Size
> 8 mm : → **0 %**

From Kim *et al.*, Neurology 2006

➡ **Recanalization mainly depends on thrombi location, length and composition**

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Why animal trials failed to select drugs?

- **Pitfall 1: brain differences**
  - Rodent have small, lissencephalic brains, with low white matter content
  - Human have large, gyrencephalic brains, with large white matter content
  - **Solution => use larger species than rodents**

- **Pitfall 2: outcomes are qualitatively different**
  - Preclinical studies rely on infarct size or cellular level measurements to evaluate therapeutic efficacy
  - Clinical trials rely on behavioral outcomes (neurologic state 90 days post stroke)
  - **Solution => focus on behavioral outcomes**

Why animal trials failed to select drugs?

- **Pitfall 3: outcomes at early times are not reliable in humans**
  - Preclinical studies have relied on early outcomes
  - Whereas clinical trials rely on late assessments (neurologic state 90 days post stroke)
  - **Solution : increase duration of recovery follow-up**

- **Pitfall 4: experimental models are homogeneous, human stroke is heterogeneous**
  - rt-PA induce 100% recanalization rate in models => homogeneity and control
  - rt-PA induce 20% full recanalization rate in humans => clot locations, constitution, duration, genetic variability, sex, age,…
  - **Solution: increasing variability and group size**

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Why animal trials failed to select drugs?

- **Pitfall 5: comorbidities**
  - Animals are young healthy males
  - Human patients are aged, with comorbidities, with sex differences
  - **Solution: use aged animals with comorbidities in both sexes**

- **Pitfall 6: therapeutic window**
  - Animals are usually treated at early time to maximize drug effect in small groups
  - Humans are often treated lately (up to 4.5h post-stroke for rt-PA), effect decreasing with time
  - **Solution: relevant treatment scheme according to mechanism of action**

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What about NAM for stroke studies?

- Must of the identified pitfalls cannot be avoided by NAMs
- Currently, no *in vitro* system can reproduce the complex interaction between all the systems involved in stroke:
  - Blood and functional arteries
  - BBB
  - Neurons + all glial cells
  - Plexus choroids and CSF flow
  - Functional immune system
  - Brain plasticity
  - …
- New innovative animal models seems to present increased translational values

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Conclusion

- We are at the very beginning of a long process to replace animals in the field of complex neurologic diseases
- NAMs such as OoC or organoids are very promising tools to study neurodegenerative diseases
- NAMs may present better translational value than animal models while allowing to strongly reduce their use in the field
- Uyltimately, NAMs may not be able to fully replace animal models in neurology, especially for pathologies were size and/or complexity matter (e.g. stroke) or with poorly understood etiology (e.g. schizophrenia)

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