Modelling Alzheimer's Disease:

A High Throughput-Compatible Assay for Detecting Tau Aggregation Using iPSC-Derived Cortical Neurons

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Neuroscience R&D
Janssen Pharmaceutica

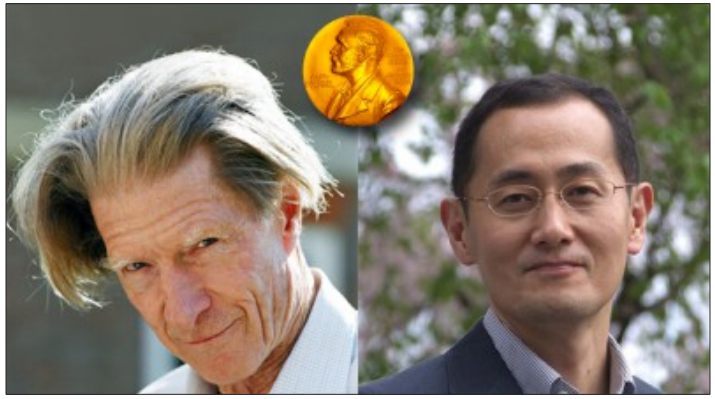
EBiSC
European Bank for induced pluripotent Stem Cells

janssen | PHARMACEUTICAL COMPANIES OF Johnson&Johnson

Outline

- iPSC and related initiatives in Europe: EBiSC
- Alzheimer’s Disease & tau
- Modelling tau aggregation using iPSCs

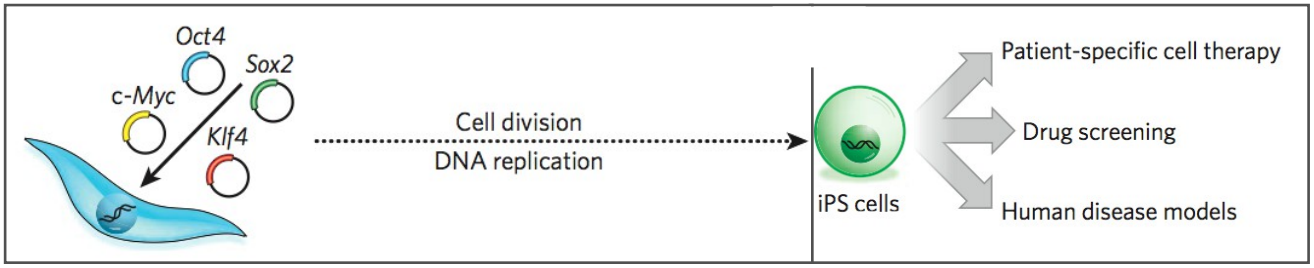
Induced Pluripotent Stem Cells: *iPSCs*



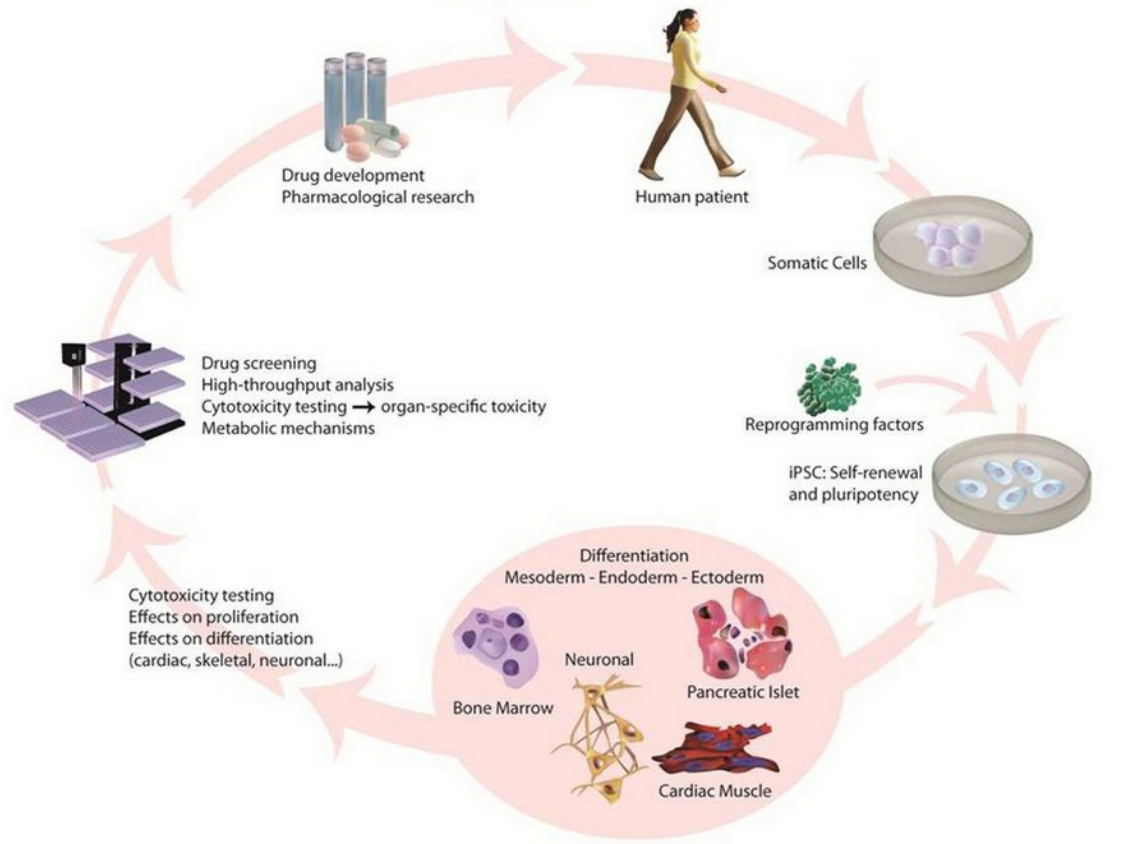
John B. Gurdon S. Yamanaka

Nobel Prize in Physiology or Medicine 2012

*"for the discovery that mature cells can be **reprogrammed** to become pluripotent"*



A virtually unlimited source of human cells available for drug discovery



Non-embryonic and non-tumoral

European Bank for induced pluripotent Stem Cells

EBiSC
European Bank for induced pluripotent Stem Cells

Creating a self financing stem cell repository for Europe

The EBiSC - European Bank for induced pluripotent Stem Cells project has received support from the Innovative Medicines Initiative Joint Undertaking under grant agreement n° 115582, resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies' in kind contribution. www.imi.europa.eu

efpia
European Federation of Pharmaceutical Industries and Associations

imi
Innovative Medicines Initiative

What is the EBiSC project?

A €35 million, IMI funded project
through which 26 leading European organisations
will establish a central facility
for the collection, testing and distribution
of iPS cells to researchers.

EBiSC At a Glance

http://www.ebisc.org/

- Pfizer co-ordinates
- Roslin Cells Ltd manages
- International SAB/EAB
- 3 yr. duration

- Total Project Value €32M
- IMI Grant of €22M

- 6 EFPIA members
- 6 SMEs
- 8 Universities
- 5 public agencies
- 1 charity funded institute

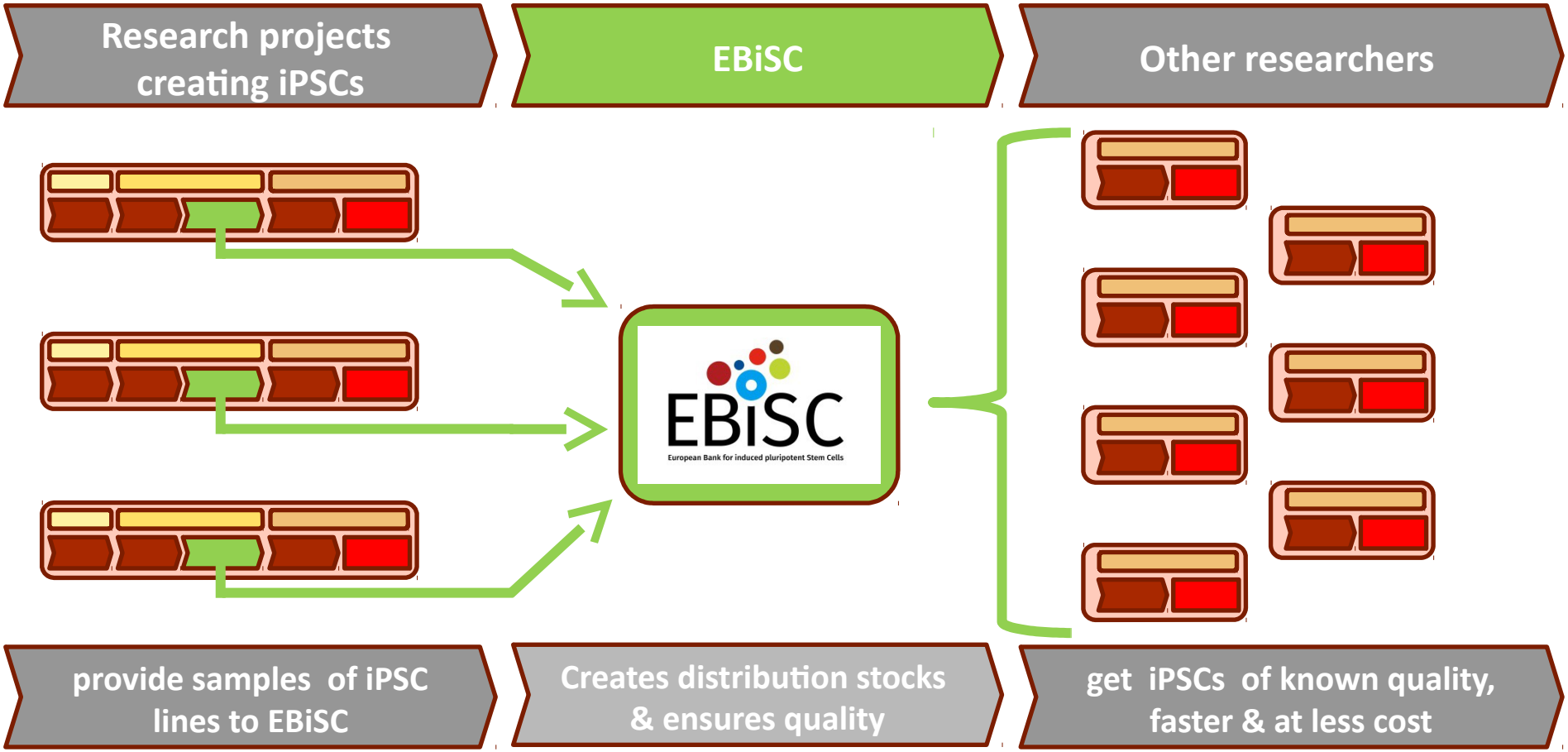
Instituto de Salud Carlos III

Establish a centralized activity in EU for the collection, testing and distribution of iPS lines

Centralized activity which is not for profit & self sustaining

Why create EBiSC?

With EBiSC: better use of research assets



What will EBiSC do?

EBiSC : improving the research landscape in Europe

Research projects creating iPSCs

EBiSC

Other researchers

Establish **central facilities** which use best cell culture technologies to operate at scale

Consent forms & **contracts** which meet needs of all stakeholders

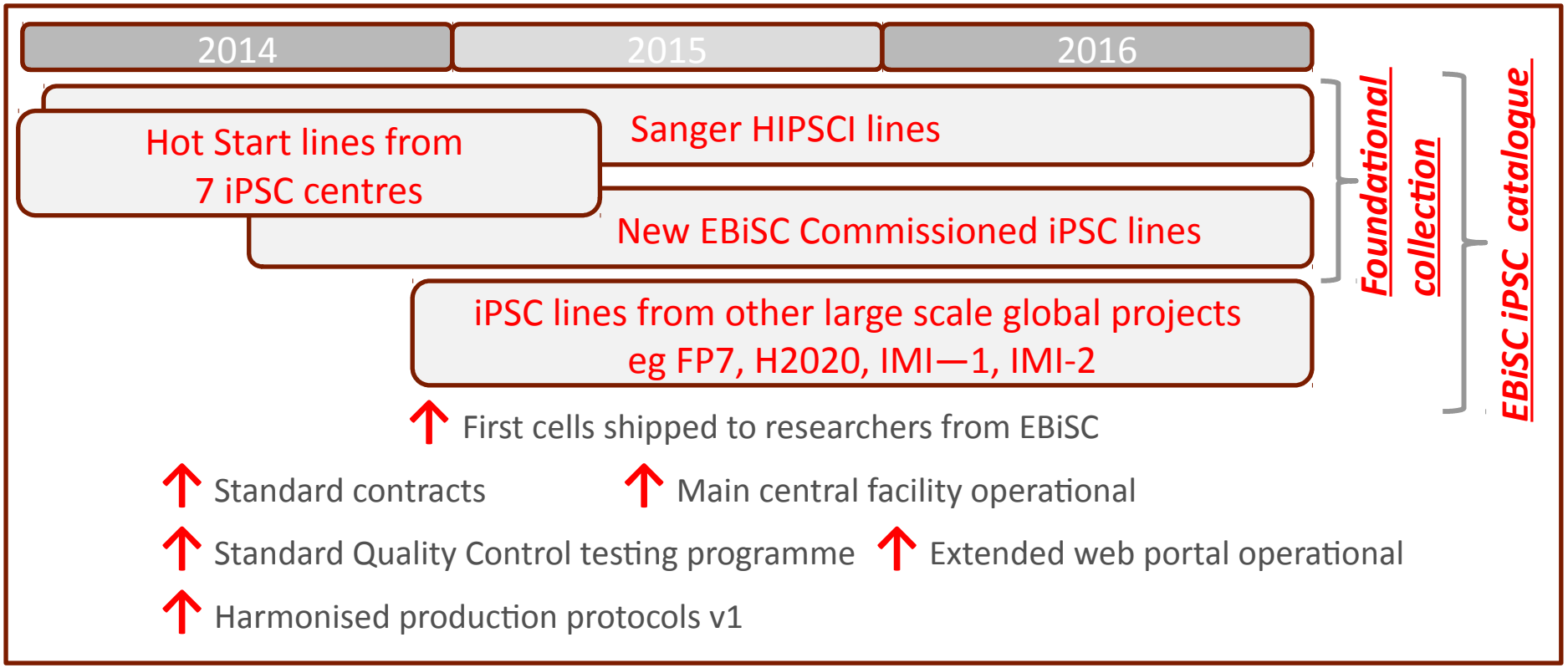
EBiSC
European Bank for induced pluripotent Stem Cells

Create a **catalogue** of cell lines which meet user needs

Common standards for processing and testing cell lines

Data management system which provides extensive data to users but controls access

When will EBiSC deliver results?



- **By end 2016:** validating working pipeline: reception, QC/banking, expansion, distribution and phenotyping
- **Beyond 2016:** expand catalogue to meet user demand leading to a self financing operation by 2019.

More about EBiSC…

Contact us at: ebisc@eurtd.com

Visit our website at: www.ebisc.eu

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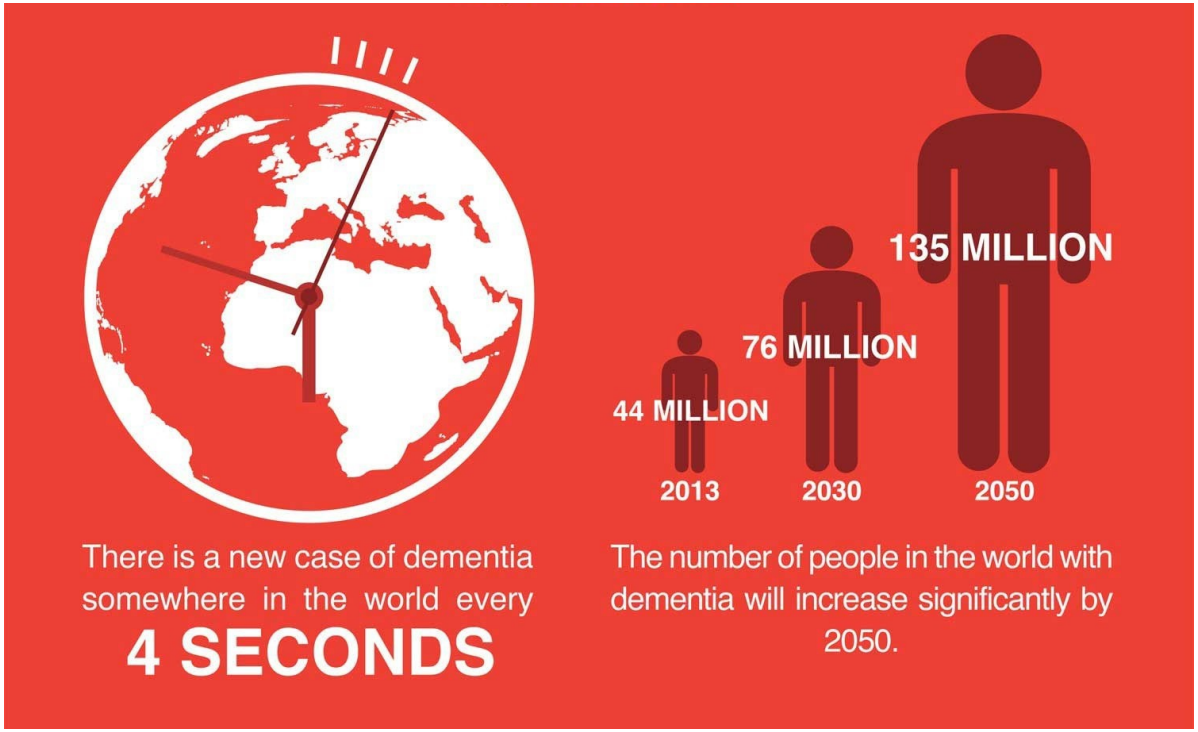
- Alzheimer’s Disease & tau
- Modelling tau aggregation using iPSCs

Dementia: one the most significant social and health crisis of 21st century

- Worldwide, **nearly 44 million** people have Alzheimer's or a related dementia: Denmark, Norway, Sweden, Belgium and The Netherlands (16.8M)

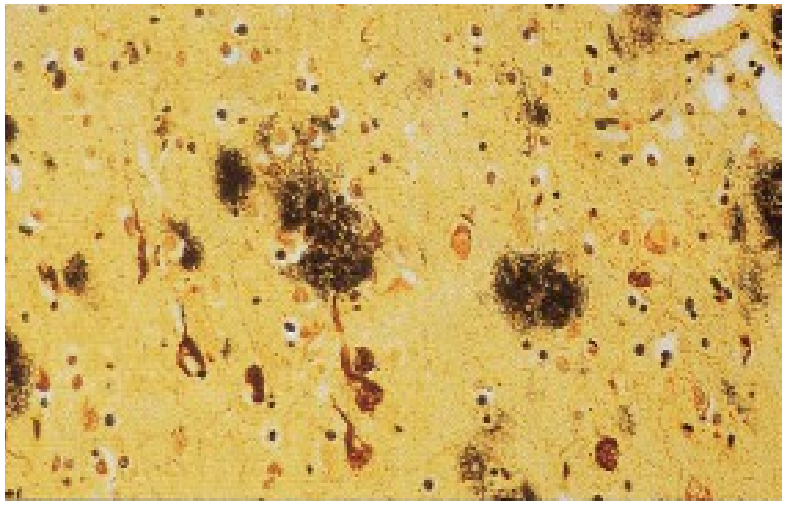
- **It cannot be prevented, cured or slowed.**



Source: ADI

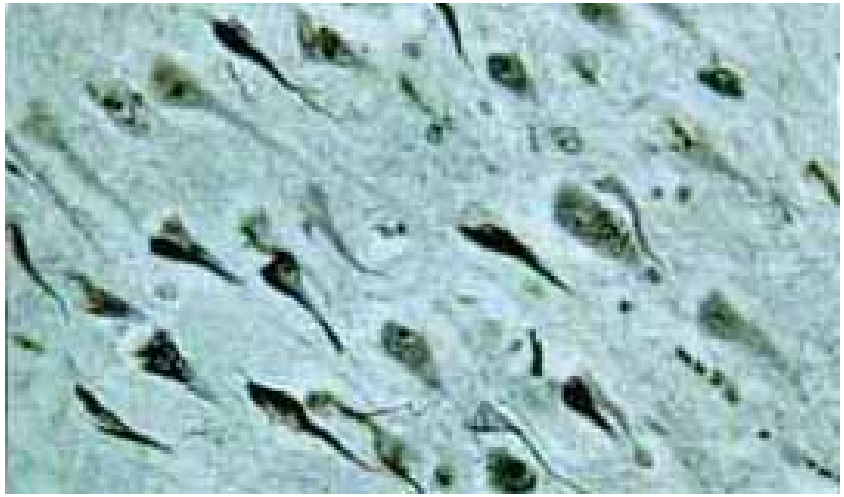
Alzheimer's Disease Pathophysiology

Amyloid plaques



"Extracellular deposits of amyloid-β peptide abundant in the cortex of AD patients"

Neurofibrillary tangles (NFTs)



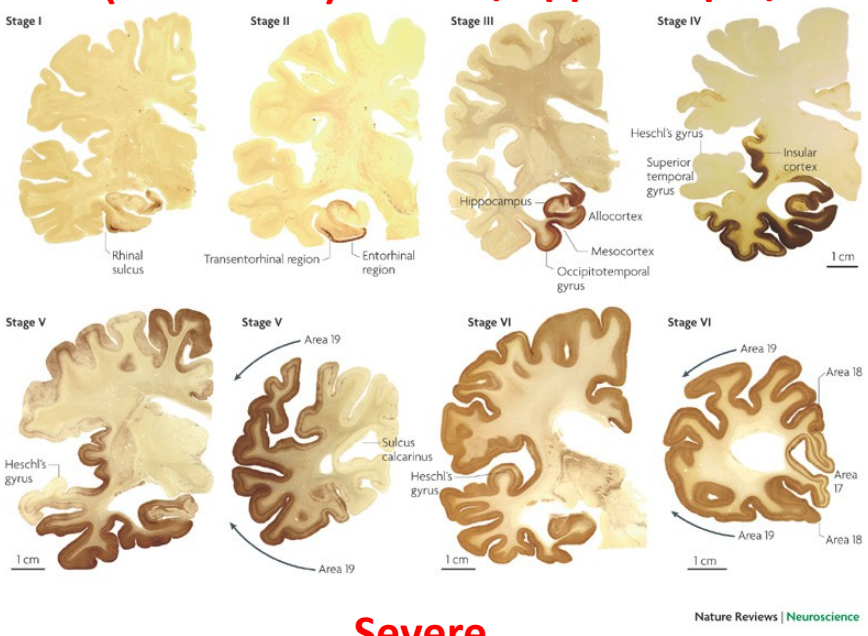
"Intraneuronal aggregates of hyperphosphorylated and misfolded tau".

High correlation between tau lesions and degree of dementia

- Braak stages: Clear **correlation** between the spatiotemporal progression of NFT pathology and cognitive state.
- NFTs, neuron loss, and synaptic loss, parallel the progression of **cognitive decline**
- Specific **genetic variants of tau** are associated with familial forms of frontotemporal dementia (FTD)

Asymptomatic (entorhinal)

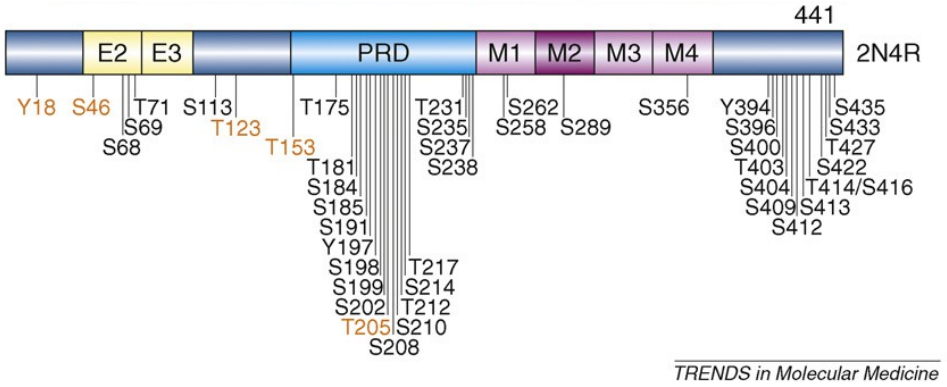
Symptomatic (Hippocampal)

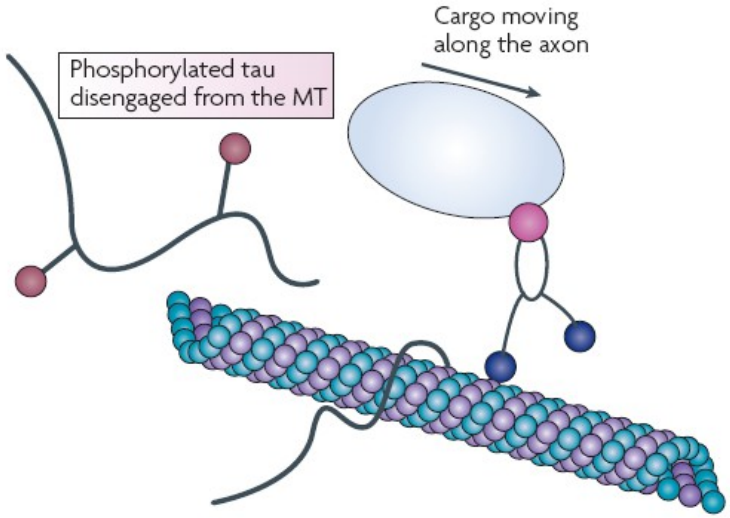


Severe (neocortical)

Tau – in normal and diseased state

- Tau is expressed in the CNS, primarily in axons of neurons
- Tau binds to and stabilizes microtubules thus ensuring proper transport of cargo to the nerve endings
- In Alzheimer's disease tau is phosphorylated by multiple kinases, detaches from microtubules and aggregates which finally leads to neuronal death
- It is hypothesized that toxic tau-species can spread from cell to cell thus "infecting" neighbouring neurons and spreading the disease

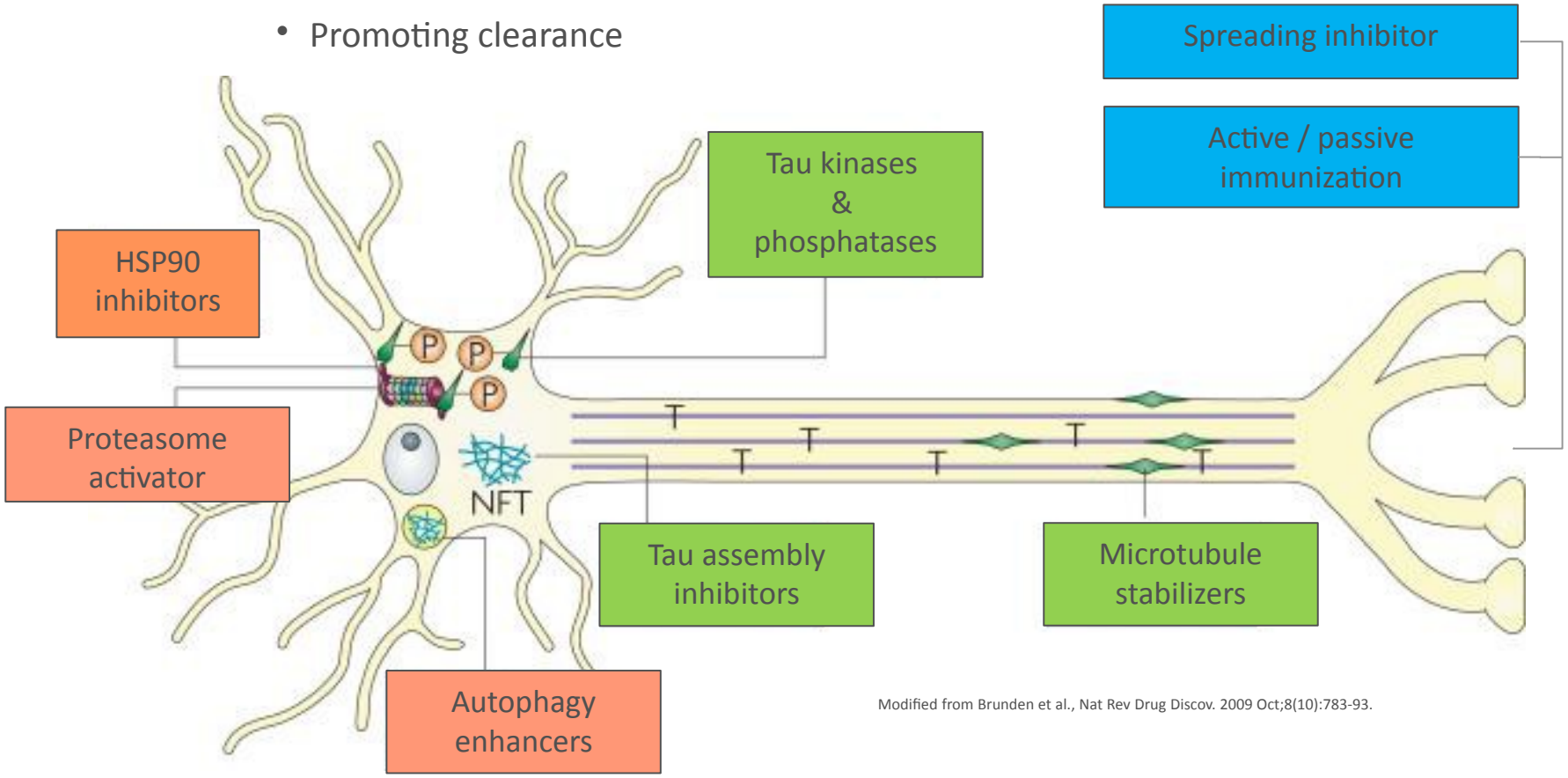




Hanger, D., et al. *Trends Mol Med* 2009.

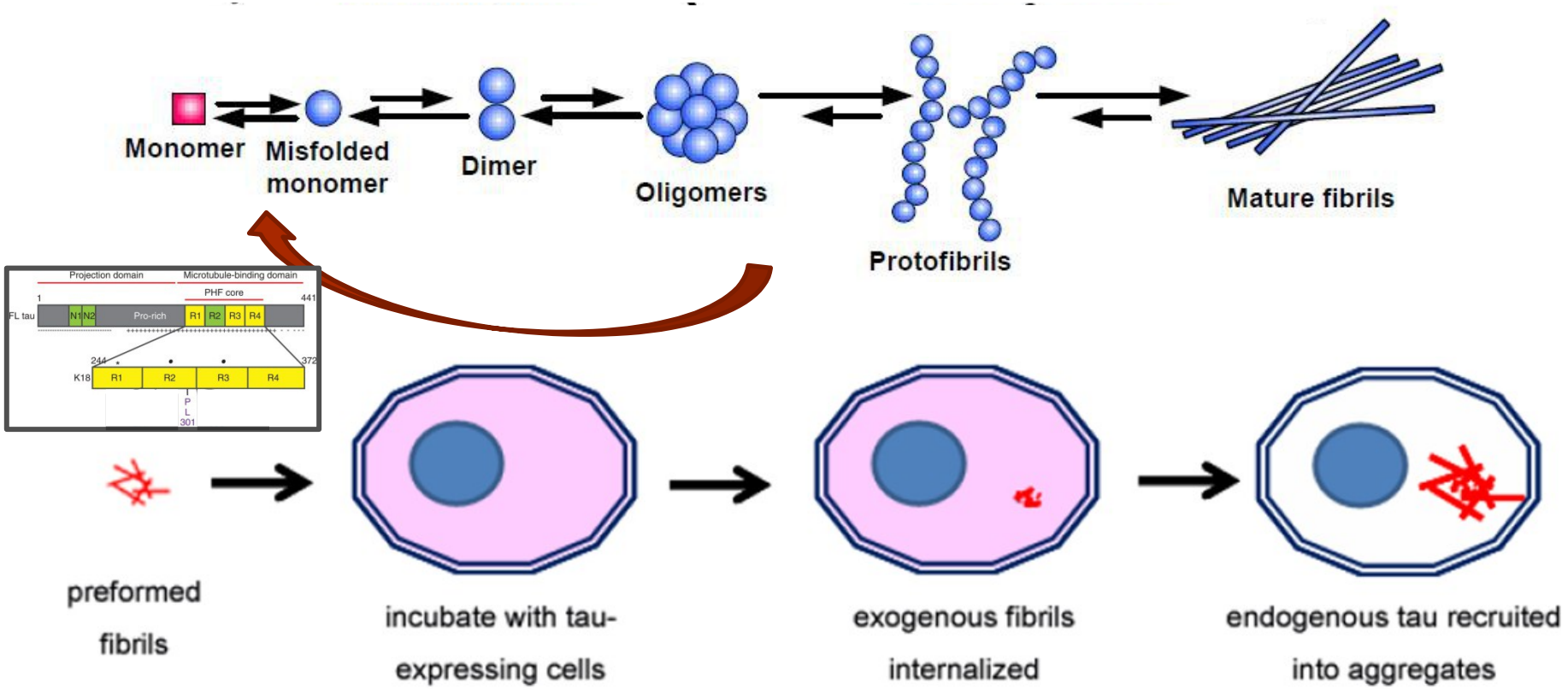
Therapeutic approaches

- Blocking aggregation
- Stop spreading
- Promoting clearance



Modified from Brunden et al., Nat Rev Drug Discov. 2009 Oct;8(10):783-93.

Tau aggregation: seeding model



- Aggregated tau induces misfolding of normal tau (seeding hypothesis).
- Evidence for seeding *in vitro* and *in vivo*

Outline

- iPSC and related initiatives in Europe: EBiSC
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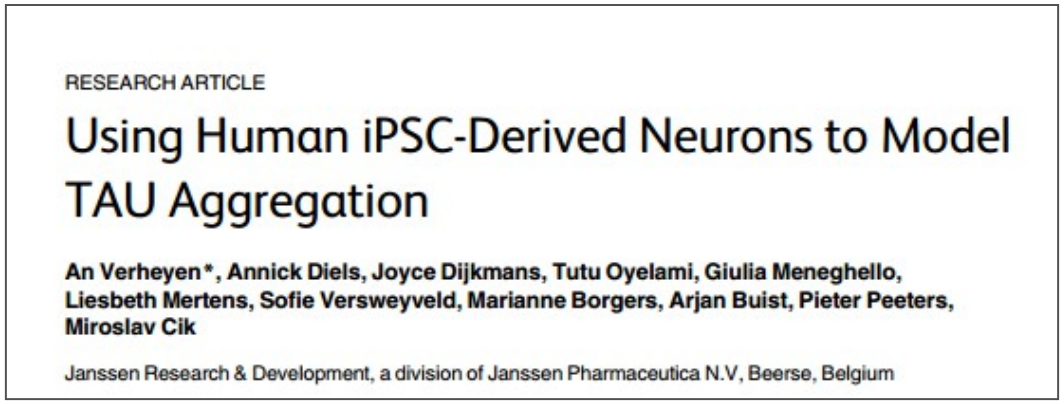
Need for a physiologically relevant/robust model to study TAU aggregation

- Human/Neuronal specific
- High reproducibility (≠primary cultures)
- Sensitive
- Scalable (high throughput)



RESEARCH ARTICLE

Using Human iPSC-Derived Neurons to Model TAU Aggregation

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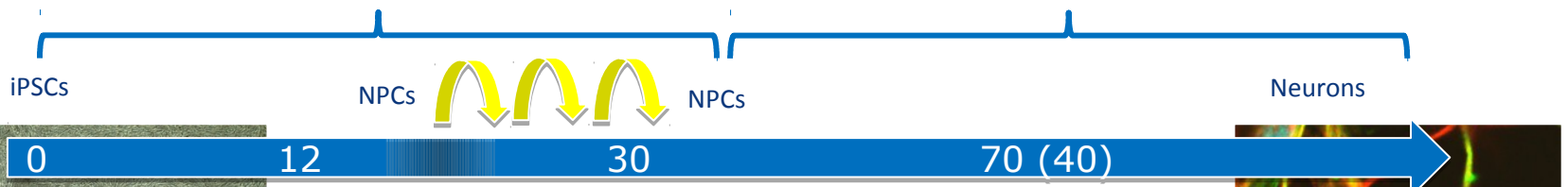
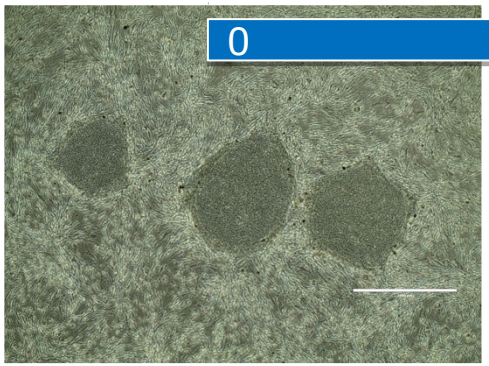
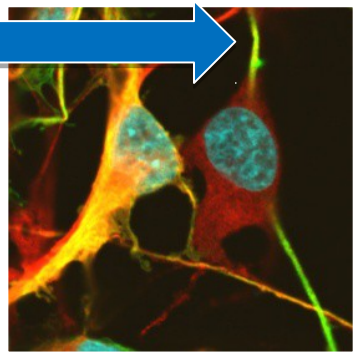
Janssen Research & Development, a division of Janssen Pharmaceutica N.V, Beerse, Belgium

Robust differentiation protocol







- Commercially available iPSC lines
- Generation of NPCs (Axol biosciences):
  - Dual SMAD inhibition 12 days
  - Freezing stocks
- Final differentiation: BDNF, GDNF, cAMP
- 384w format (HCI and HTS)

Robust culture conditions to allow standardization:

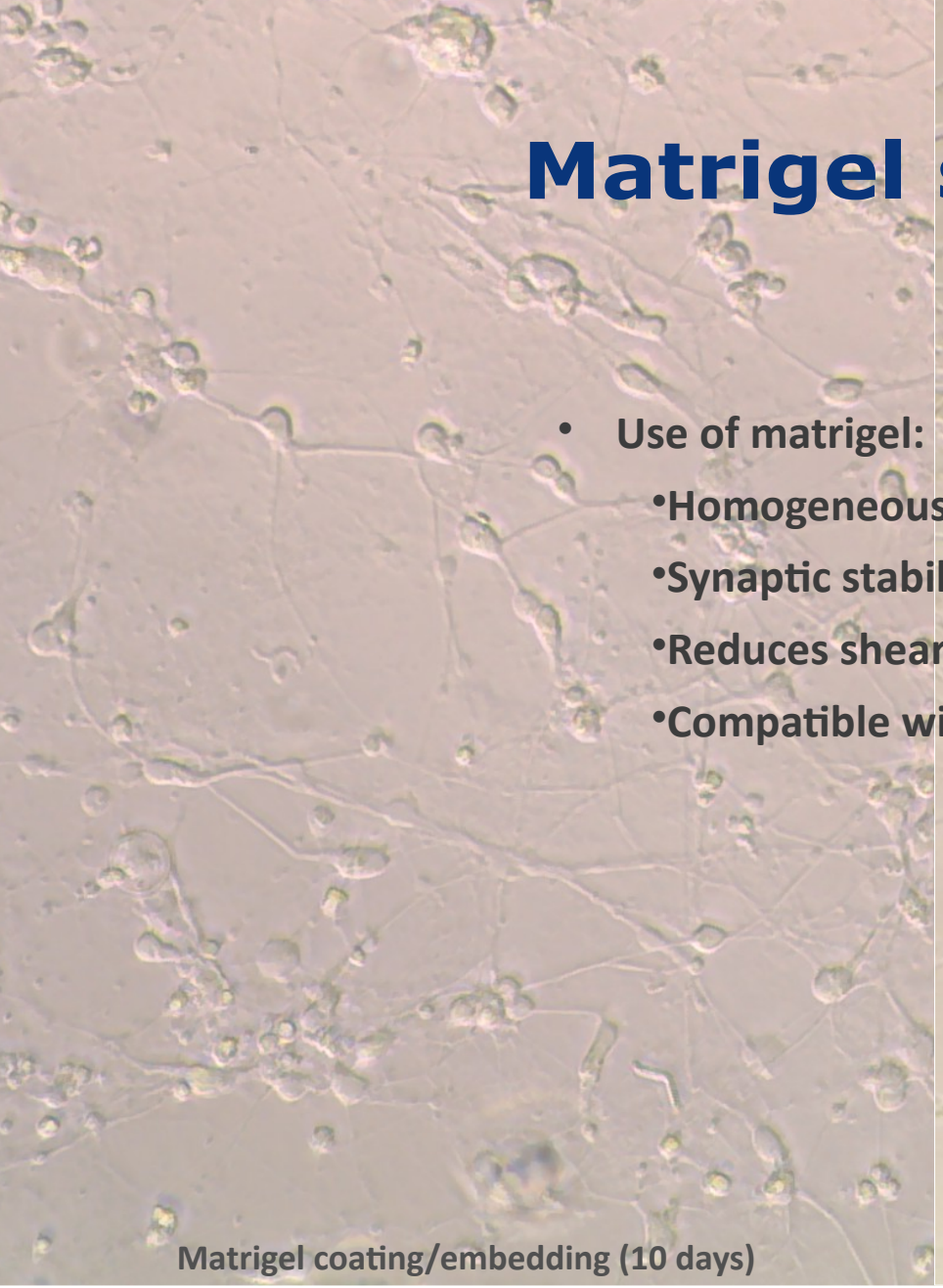
- Homogeneous cell distribution (HCI)
- Long term stability/adherence (synapse strength)
- Reduced shearing stress due to media change/plate handling
- Scalability/automation (avoid pre-coating)

- **Neurospheres/Organoids**
  - Intrinsic capacity to form in vitro a “cortical like” structure.

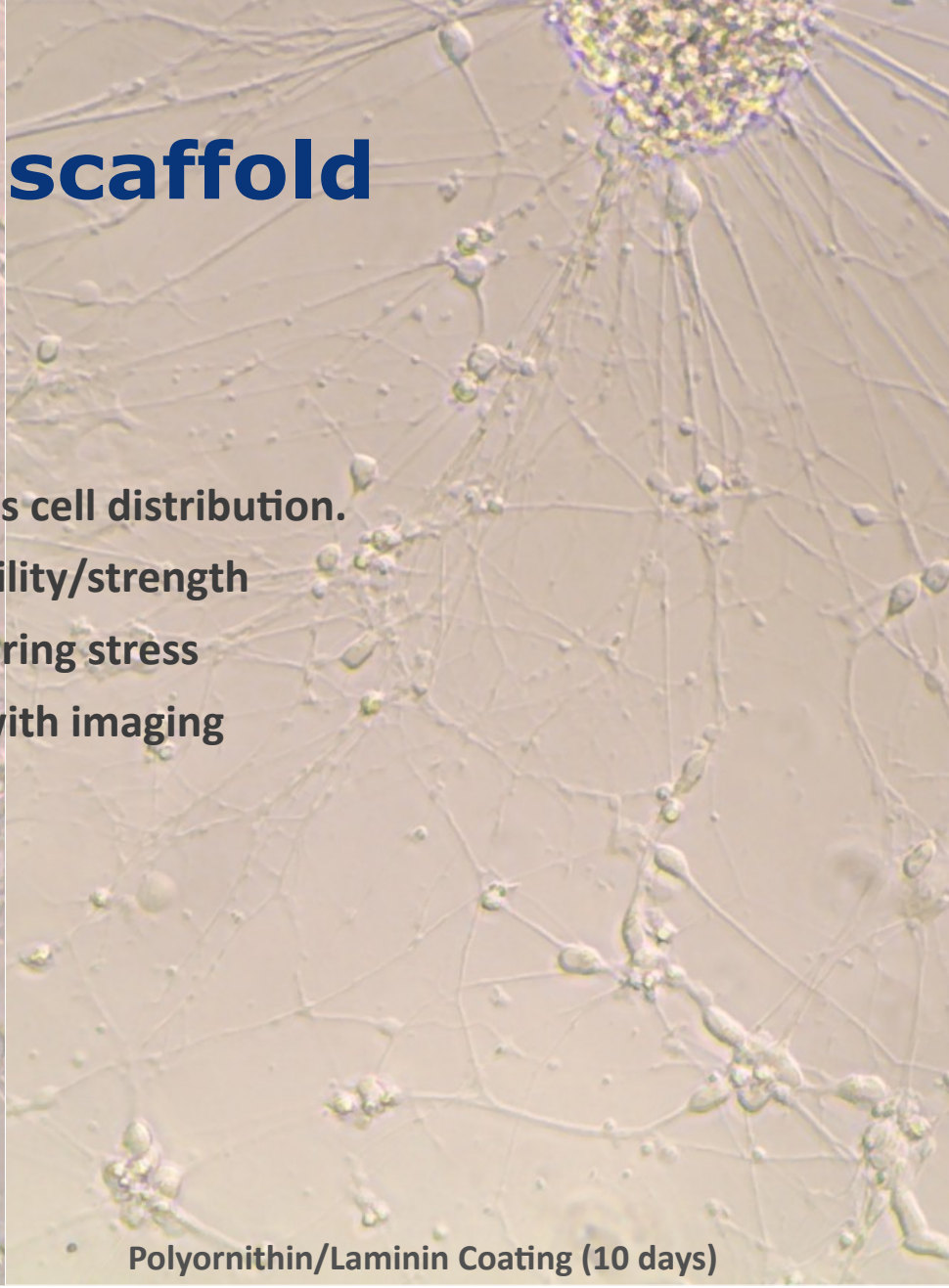
- **Scaffolding biomaterials:**
  - Matrigel ®

Matrigel scaffold

- Use of matrigel:
  - Homogeneous cell distribution.
  - Synaptic stability/strength
  - Reduces shearing stress
  - Compatible with imaging



Matrigel coating/embedding (10 days)



Polyornithin/Laminin Coating (10 days)

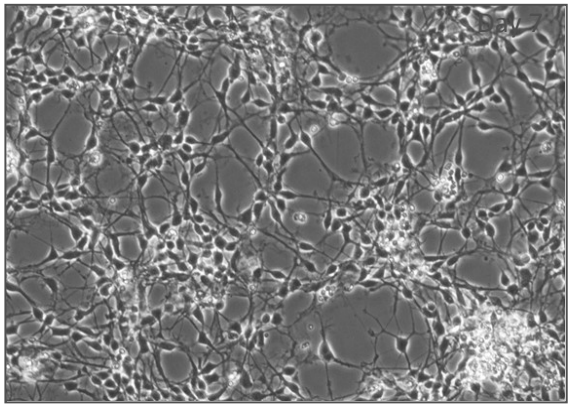
Matrigel scaffold

- **Low density matrigel:**

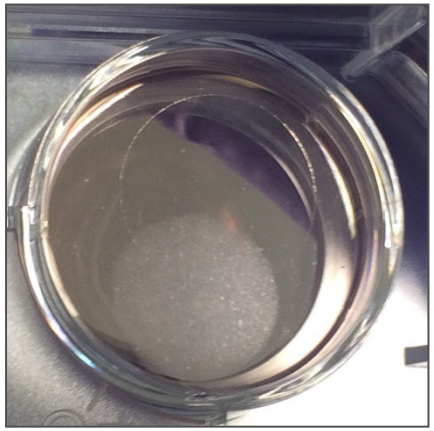
- Dilution 1:15 (MG:N2B27)
- Cells sediment before MG gels
- Cells are “coated” with matrigel
- Thickness: 50um (approx.)
- For TAU aggregation assays

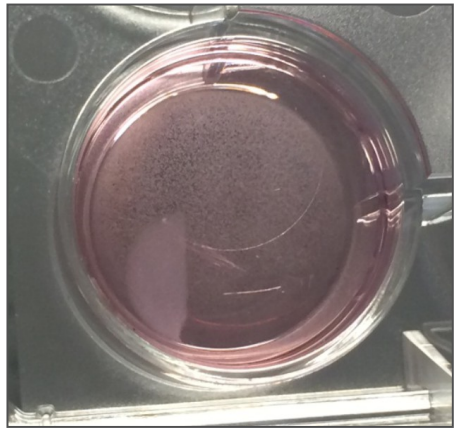
- **High density matrigel:**

- Dilution 1:1 (MG:N2B27)
- No sedimentation, fast gelling (37C)
- Cells are “embedded” in matrigel
- Thickness: 200um (approx.)
- For electrophysiology



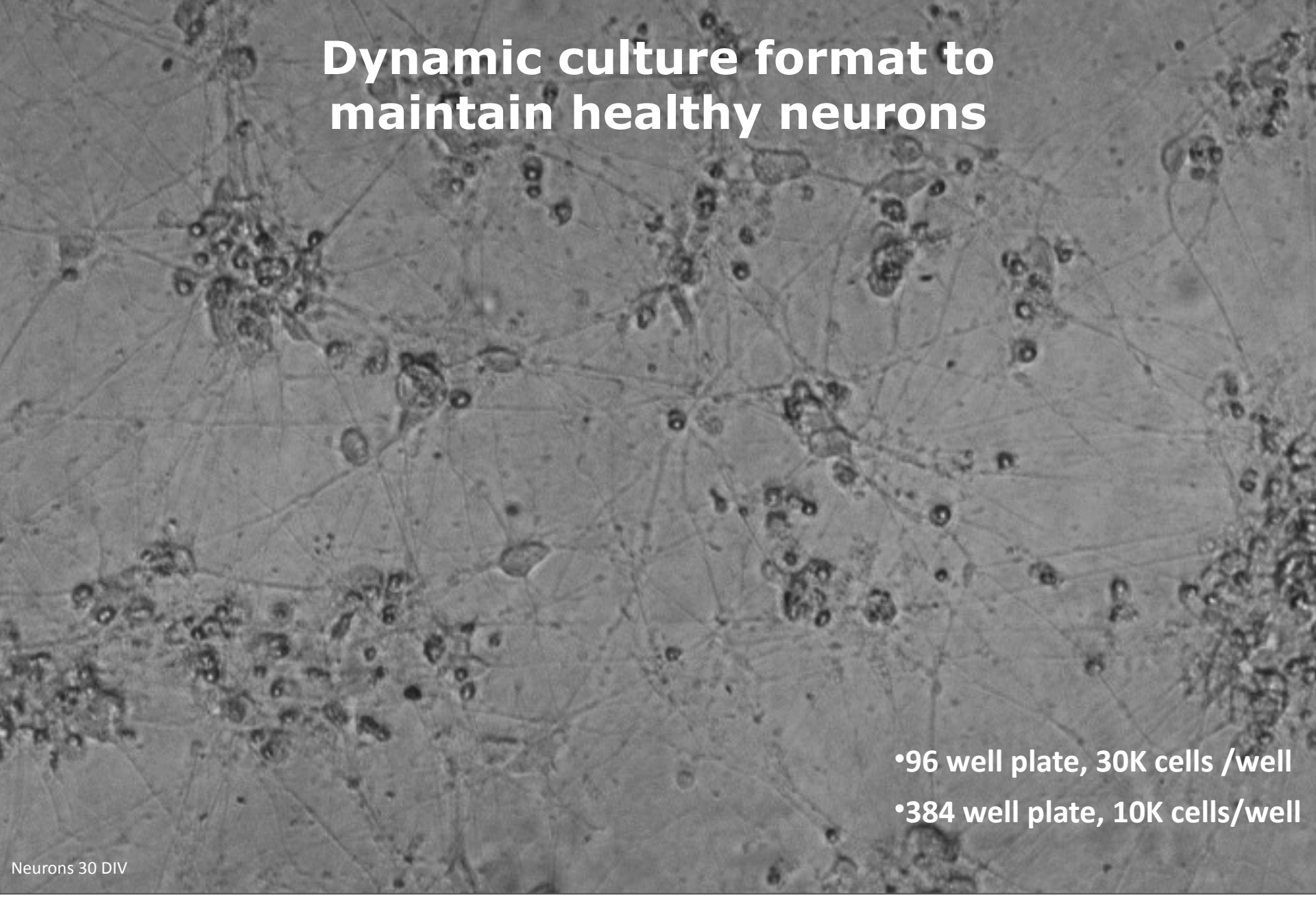
7 Days in vitro





7 weeks in vitro

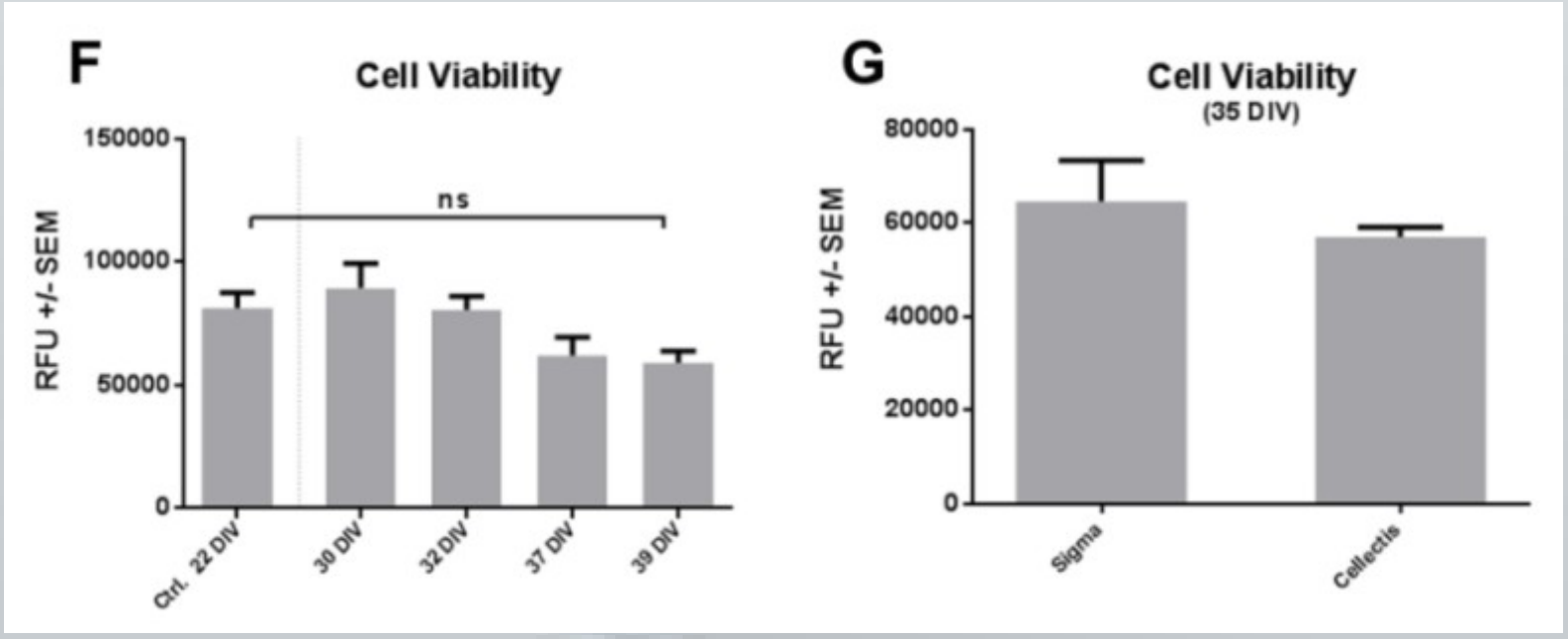
Dynamic culture format to maintain healthy neurons



- 96 well plate, 30K cells /well
- 384 well plate, 10K cells/well

Neurons 30 DIV

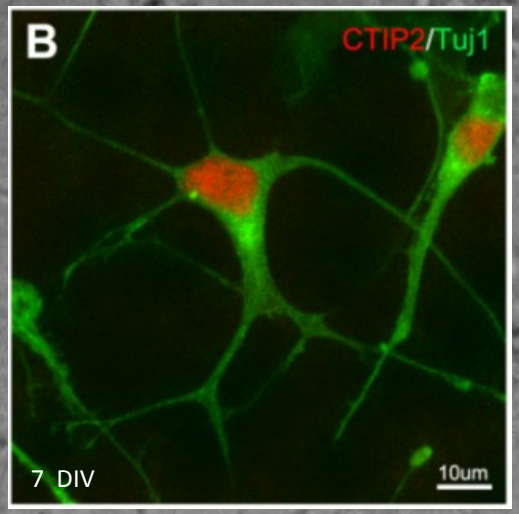
Dynamic culture format to maintain healthy neurons

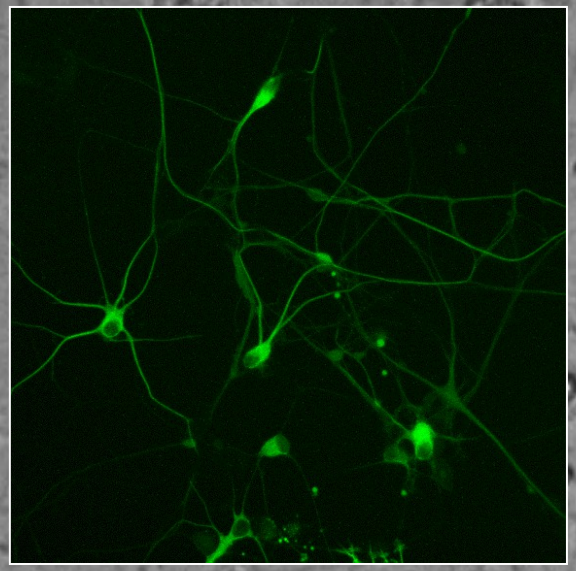


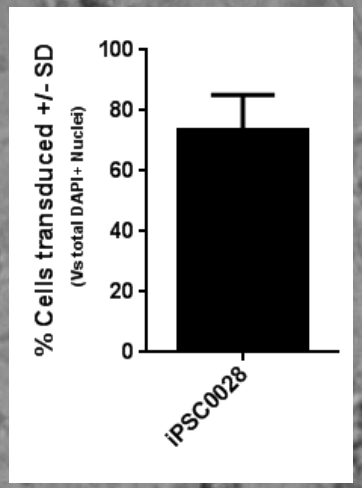
- IncuCyte ®

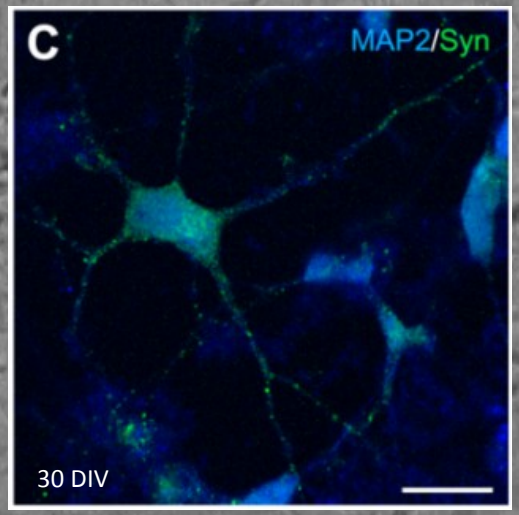
Neurons 30 D

Differentiated neurons are transducible

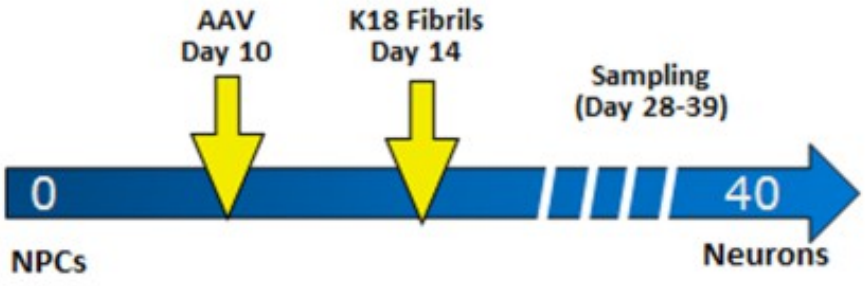


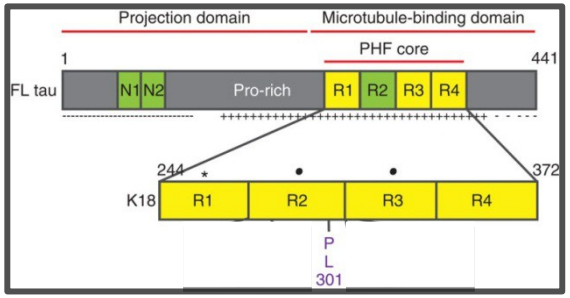






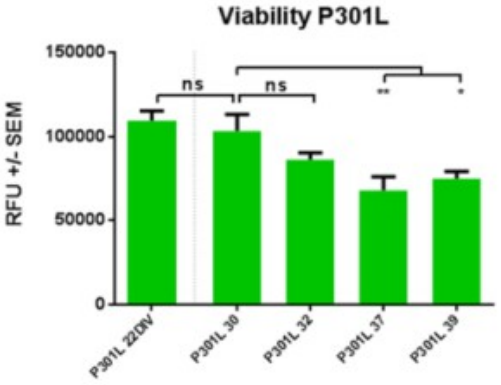
Adenoviral-mediated TAU aggregation using iPSC derived neurons

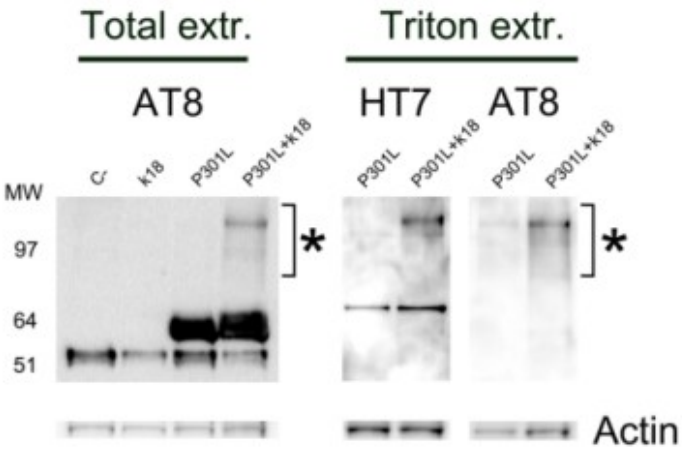


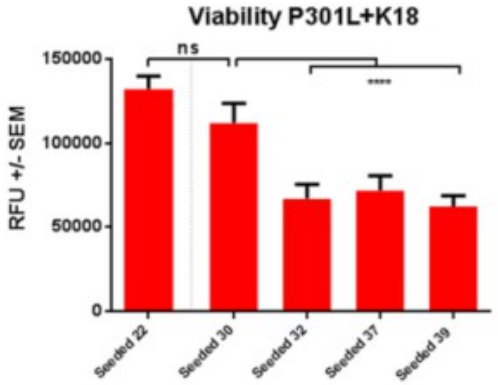


- **Experimental setup:**
  - 10k Cells/well
  - AAV P301L
  - 384WP: 75uL media, 20uL lysate
- **Conditions:**
  - No treated cells (**C-**)
  - No treated cells + K18 Fibrils (**K18**)
  - AAV P301L
  - AAV P301L + K18 (**P301L +K18**)

TAU aggregates in human neurons

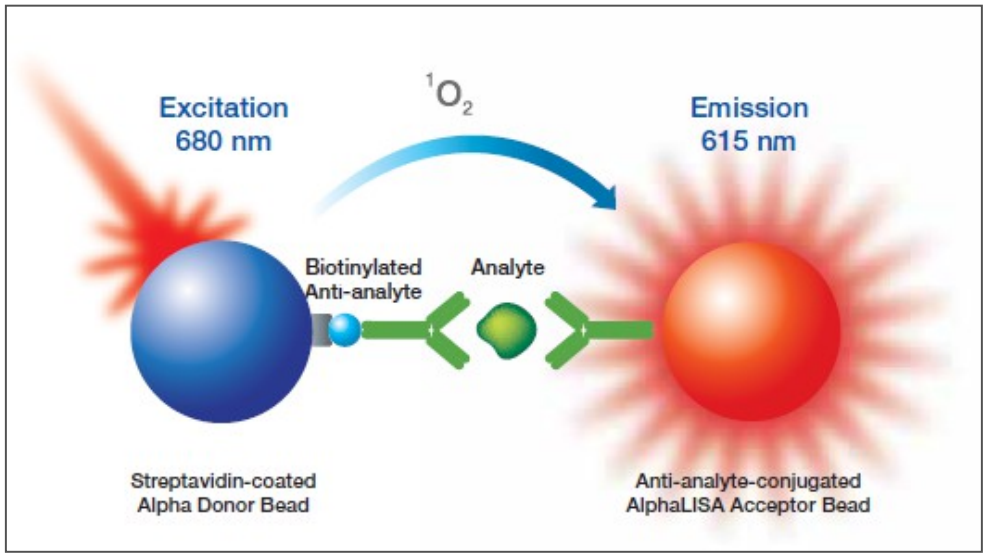


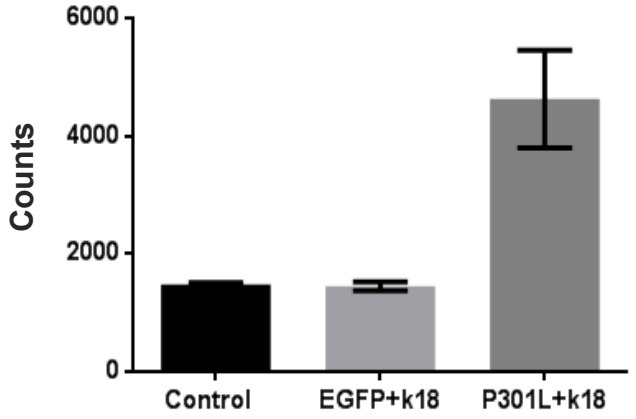




How to measure TAU aggregation? alphaLISA - TAU

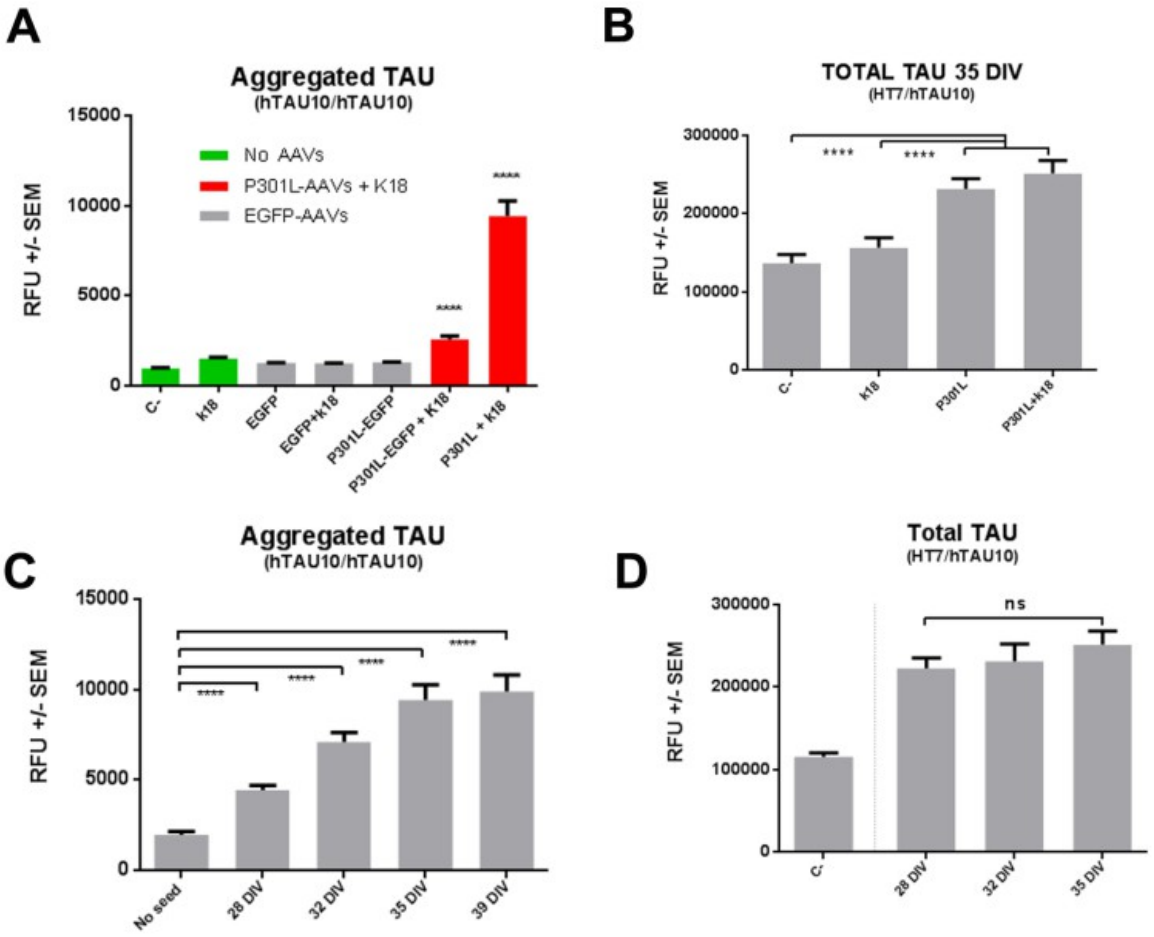
- alphaLISA is the non-wash ELISA assay alternative
- alpha = amplified luminescent proximity homogeneous assay
- 3-4 hours assay



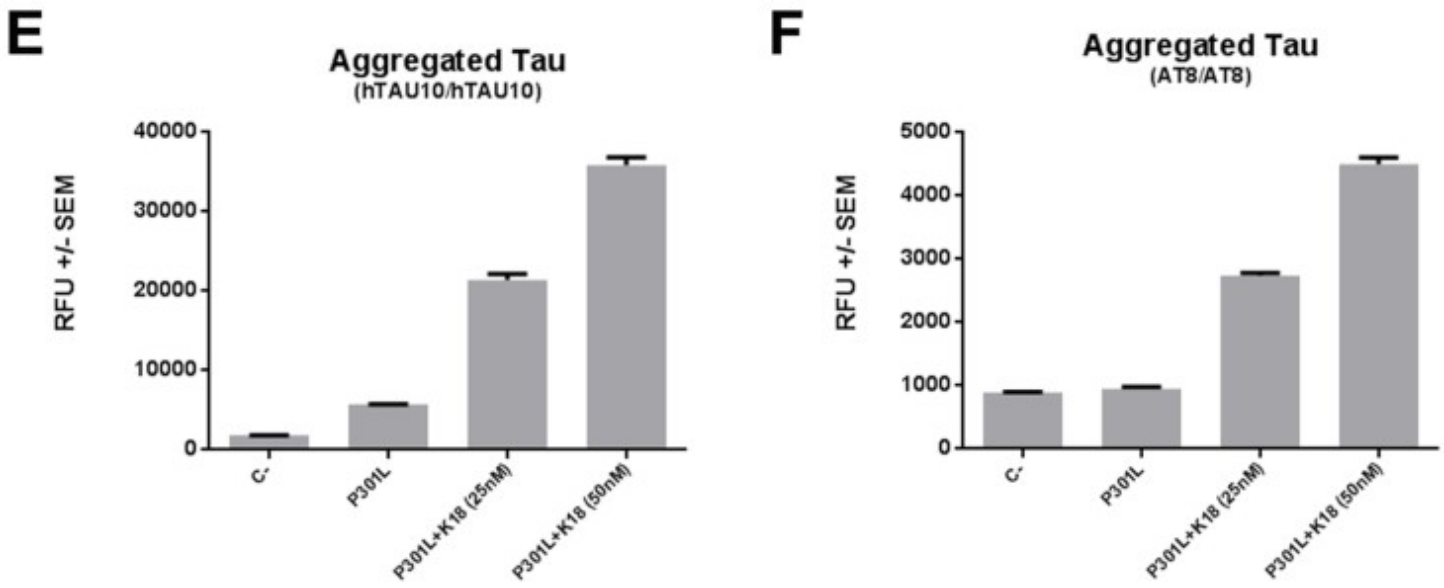


The higher the emission, the more TAU aggregation

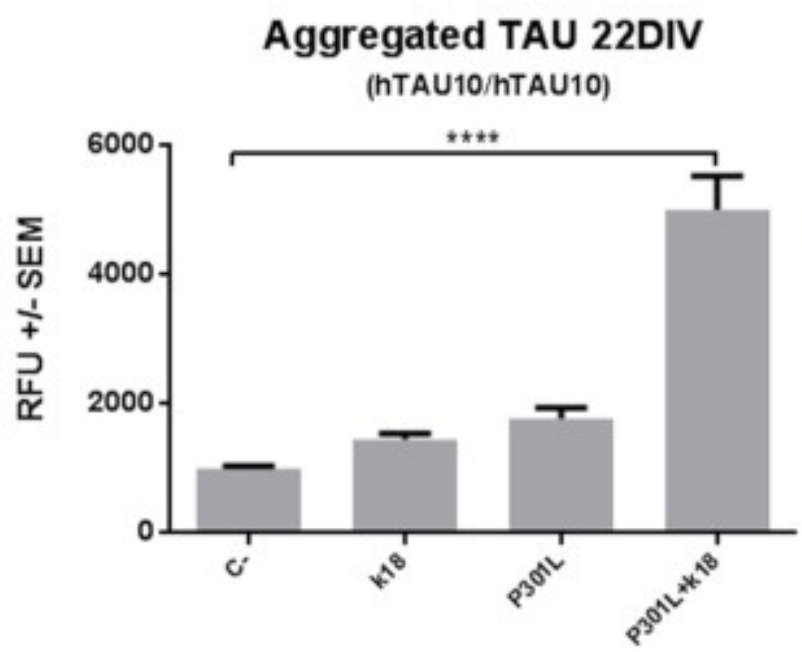
Defining baseline values and sampling time



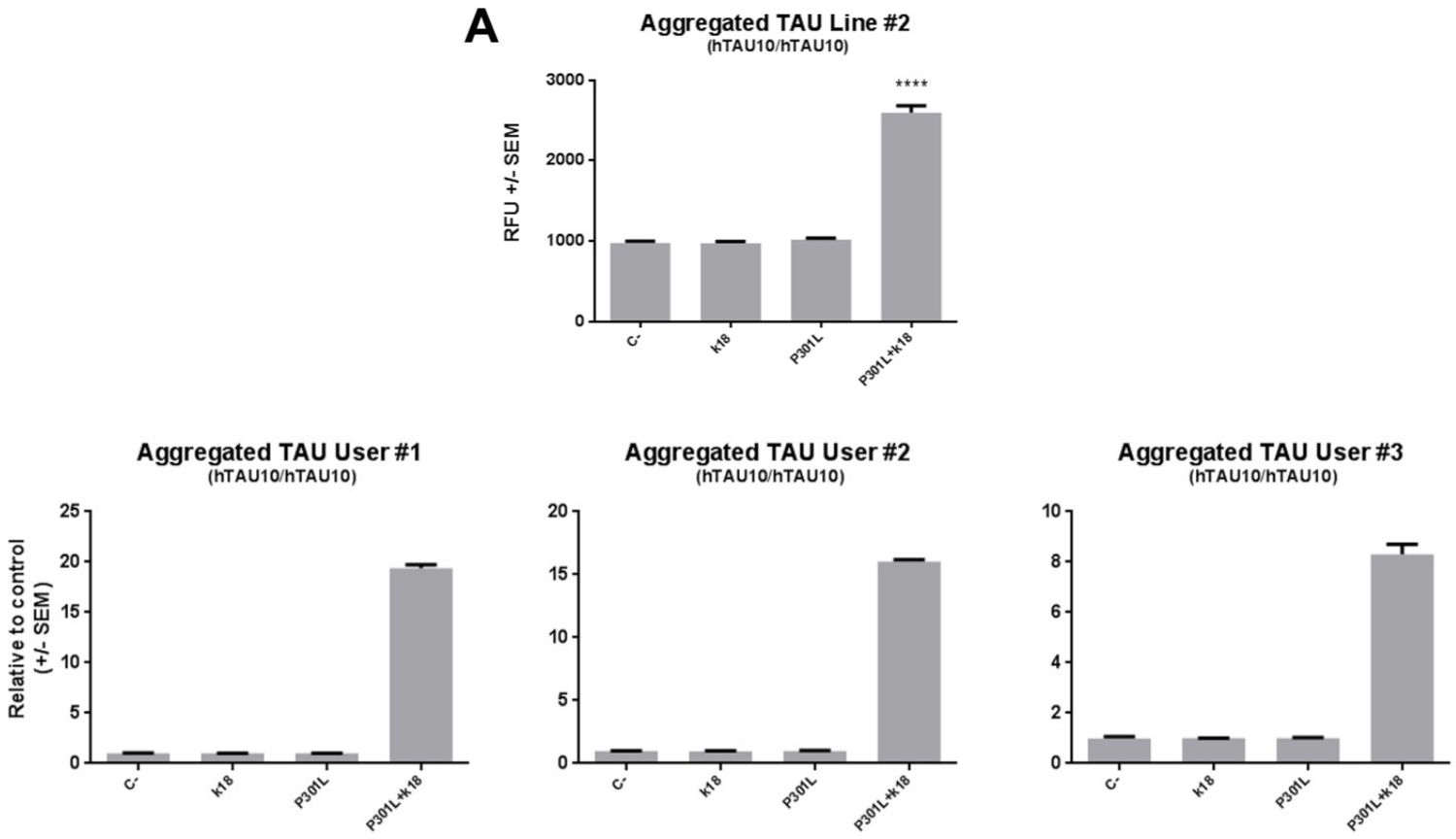
K18 Dose response (Z'=0,52)



Shortening protocol: 22 days



Assay reproducibility: across cell lines and users



New assay to study TAU aggregation

- Human/Neuronal specific ✓
- High reproducibility (≠primary cultures) ✓
- Sensitive ✓
- Scalable (high throughput) ✓

Towards the standarization of iPSC technology for drug development in neuroscience: Perspectives and challenges

1. To tackle different aspects of tau pathology: seeding, aggregation, clearance?

2. Test available mutant cell lines and isogenic controls: less artificial

3. Optimizing maturation process: mature neurons in shorter time, co-culture (astrocytes)

4. Testing chemically defined scaffolding materials: eg. hyaluronic acid

Thanks!

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- An Verheyen
- Ines Royaux
- Andreas Ebneth

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EBiSC
European Bank for induced pluripotent Stem Cells

stem BANCC
Stem Cells for Drug Discovery

Stellar
NEURODEGENERATION COLLABORATION PROJECT

imi
Innovative Medicines Initiative