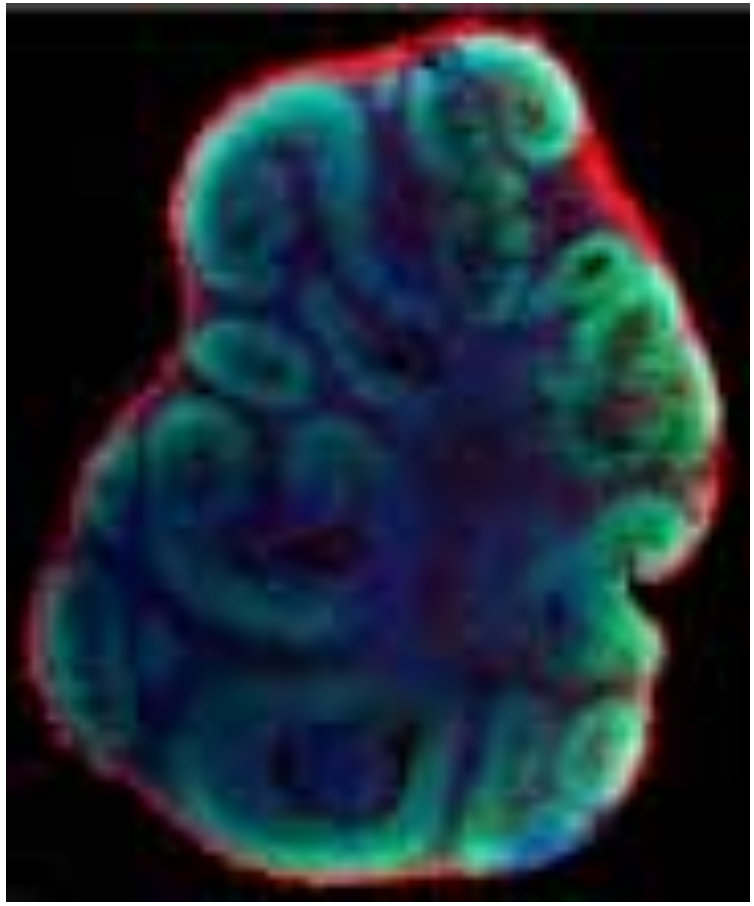
Institut Pasteur | DSCB Developmental & Stem Cell Biology

Cerebral organoids from patients with a neurodegenerative and progeroid syndrome



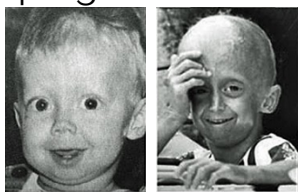
Miria Ricchetti

Les Rencontres du GDR Organoides
2nd December 2022

Progeroid diseases

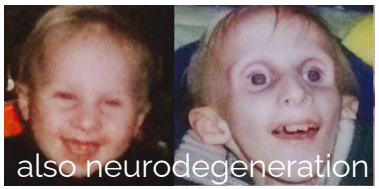
- Dramatically accelerated ageing
- **Progressive diseases**

Hutchinson-Gilford progeria (HGPS)



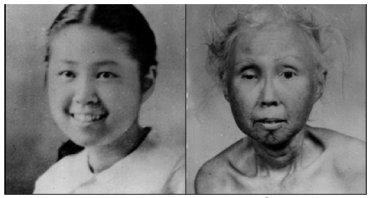
1 yr 8 yr

Cockayne syndrome (CS)



3 yr 8 yr

Werner syndrome (WS)



15 yr 48 yr

Mutations known since decades

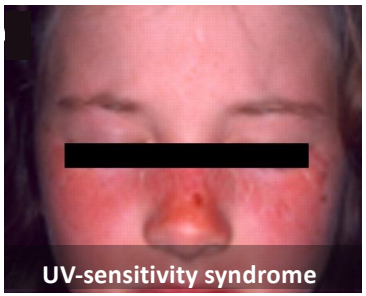
→ how do they promote ageing?

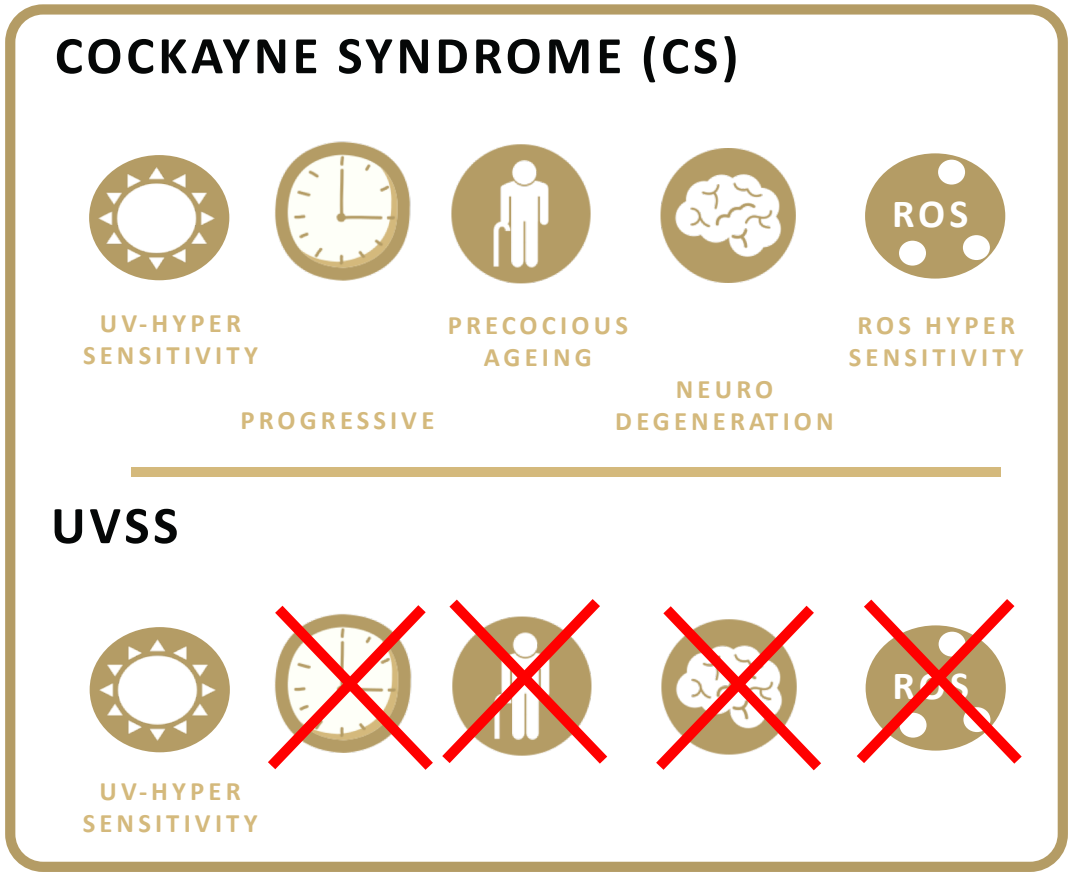
Provide clues on normal physiological ageing?

Cockayne syndrome and UVSS

mutation: *CSA* or *CSB*

mutation: *CSA* or *CSB*





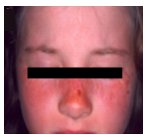
Working hypothesis

CS progeria and neurodegeneration are due to mitochondrial dysfunction and transcription reprogramming and not DNA repair defects

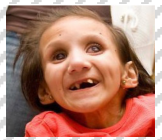
CSA and CSB are multifunctional:

- Repair of UV damage (by TC-NER)
- **Transcription**
- **Chromatin remodeling**
- In mitochondria

Strategy



UVSS

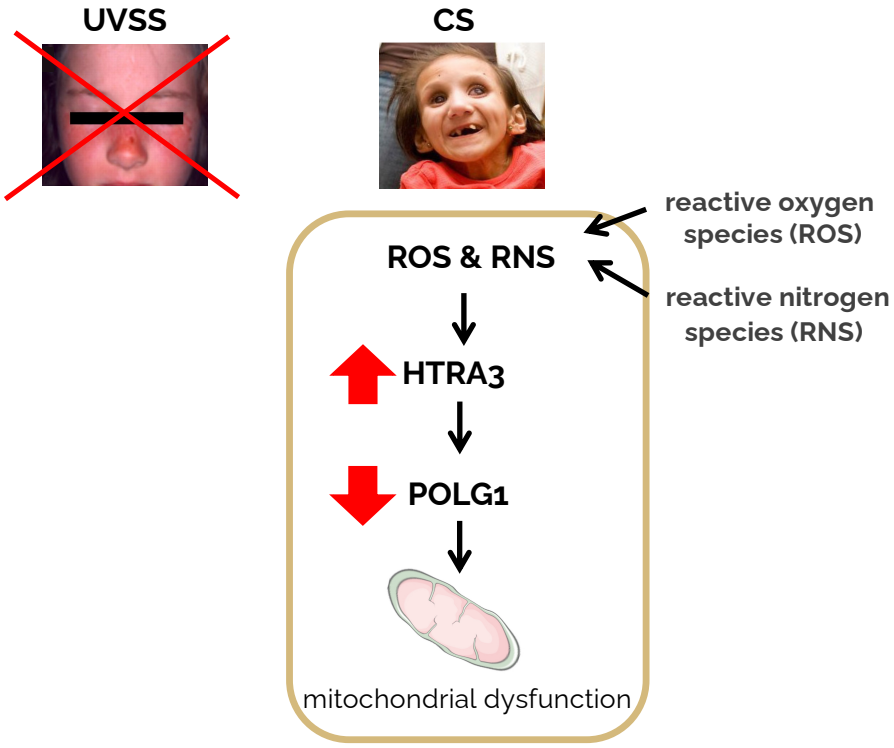


CS

(multiple patients)

Identify these defects

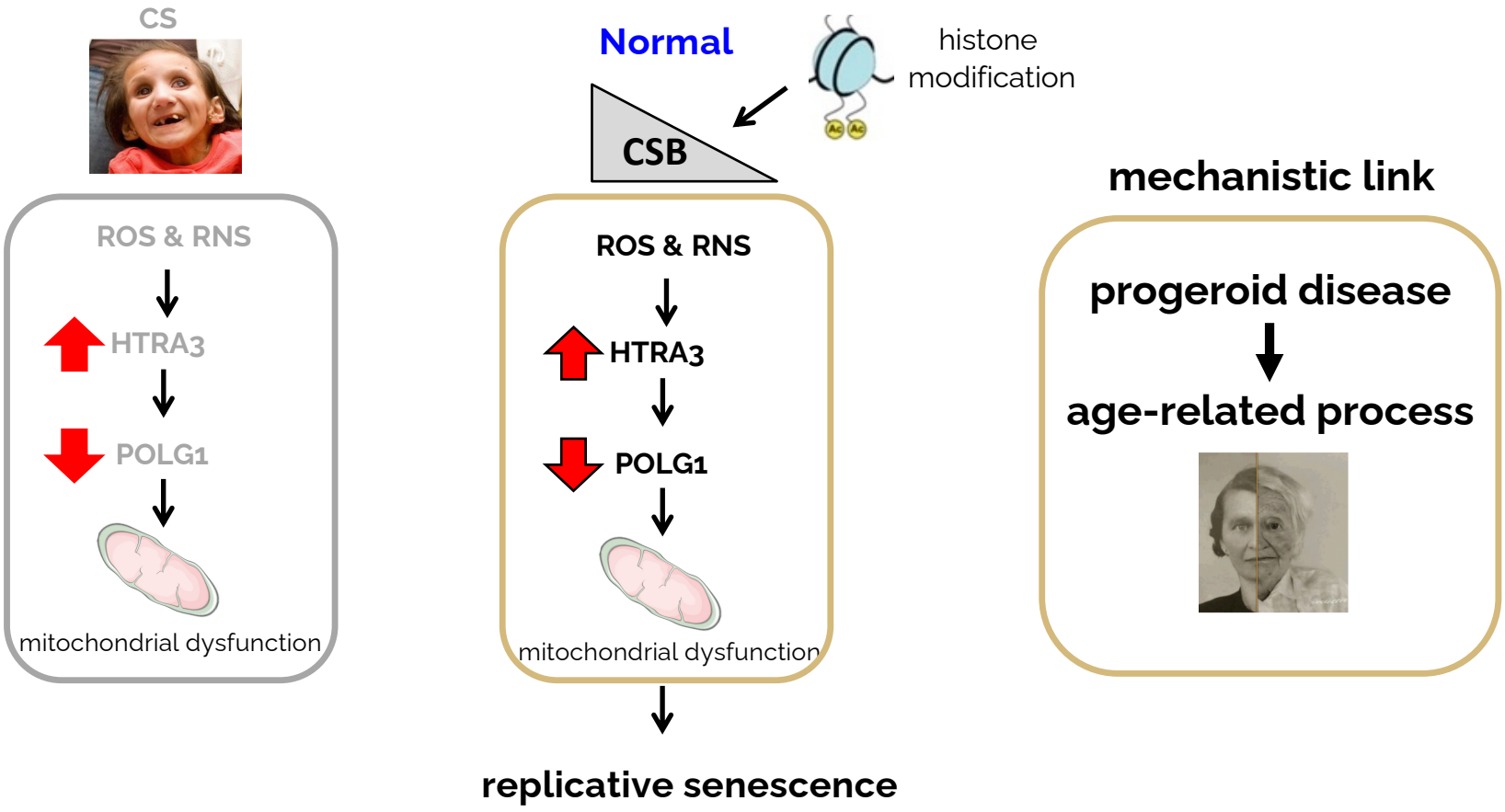
A mechanism for Cockayne syndrome



Chatre et al, PNAS 2015

- **mitochondrial dysfunction in CS**
- **mechanism identified**
- **rescue molecule (MnTBAP)**

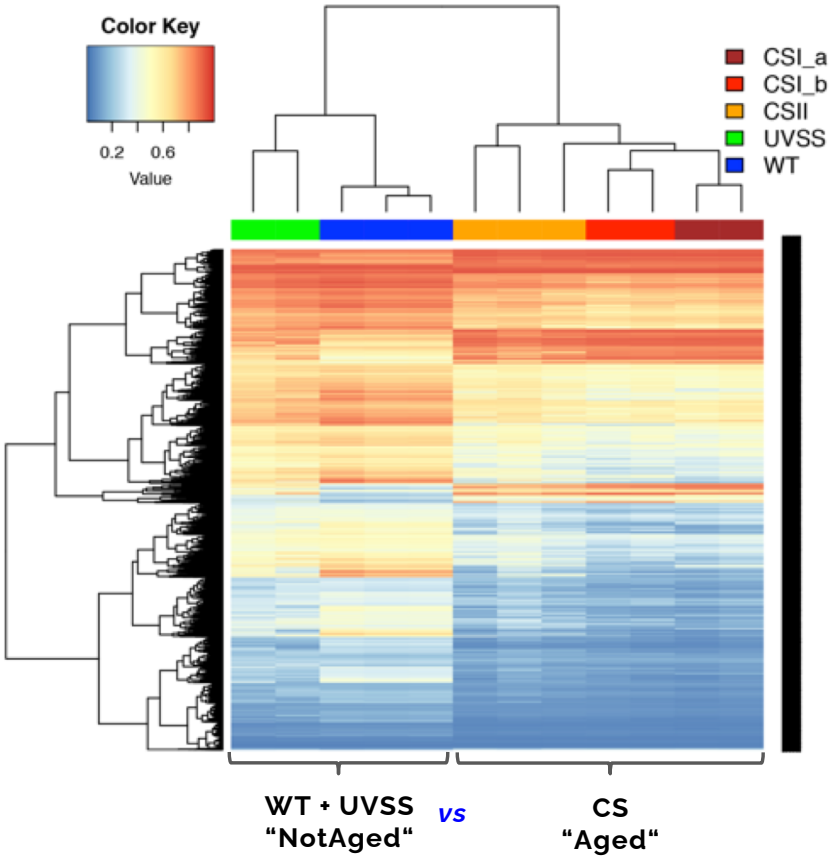
CSB depletion triggers replicative senescence



Crochemore et al, Nature Comms, 2019

CS epigenomic signature

Genome-wide DNAm in patient-derived fibroblasts



1817 DMRs
(Differentially Methylated Regions)

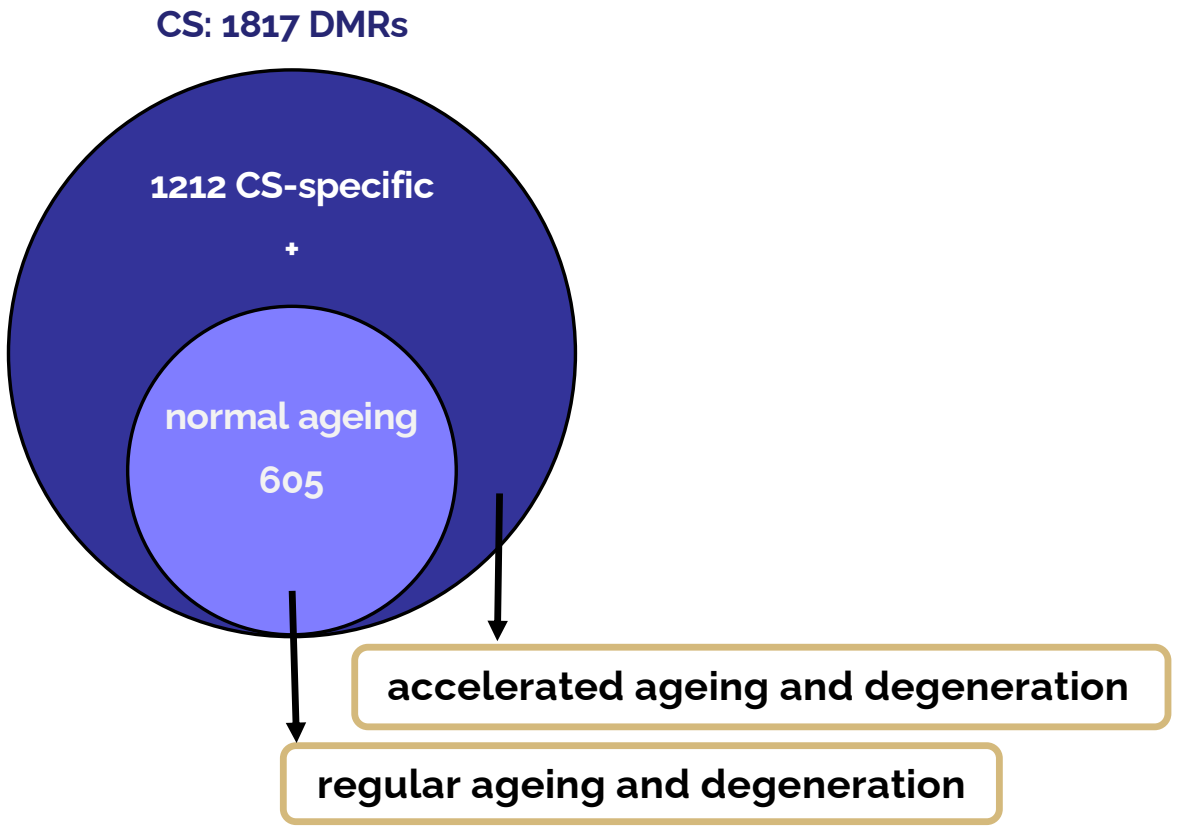
Gene set enrichment analysis:

- **Developmental transcription factors**
- **Transporters**
- **Cell-to-cell junction factors**

abundance of neural-related factors

Crochemore et al, BioRxiv 2021_445308 and in revision (collab. C. Franceschi)

CS shares differentially methylated genes with regular ageing



on selected genes:

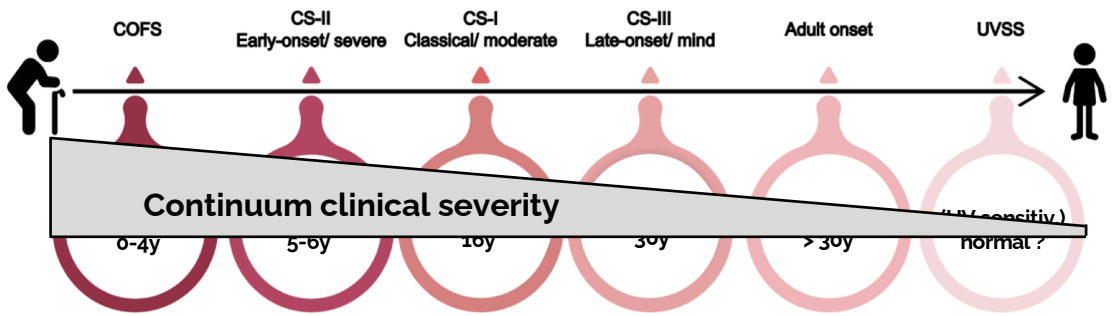
functional analysis

Overcome limitations of CS experimental models

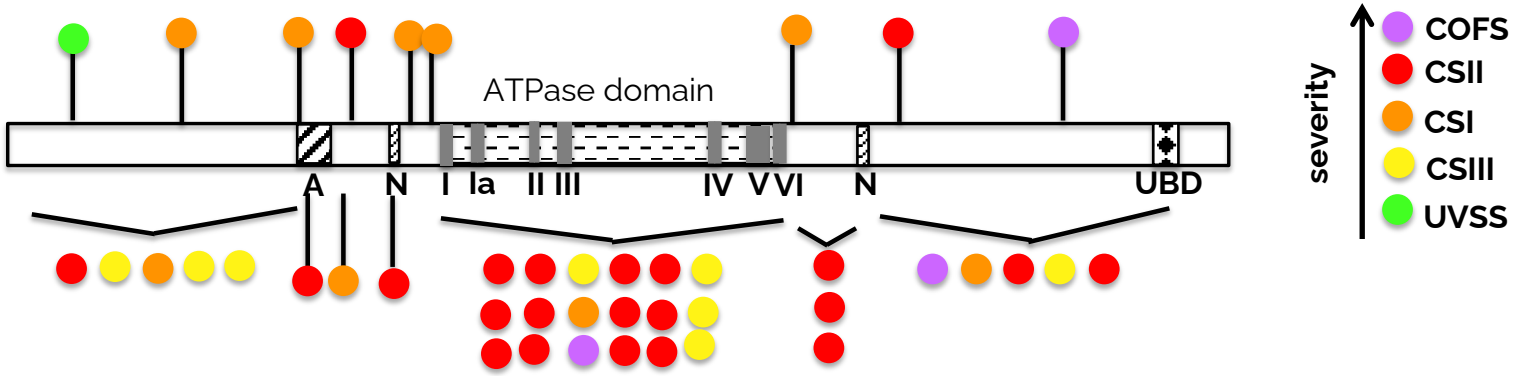
- Mouse models do not recapitulate the disease (no precocious ageing, no neurodegeneration)
- Rat models (poor genetics) not progeroid

CS heterogeneity & poor genotype/phenotype correlation

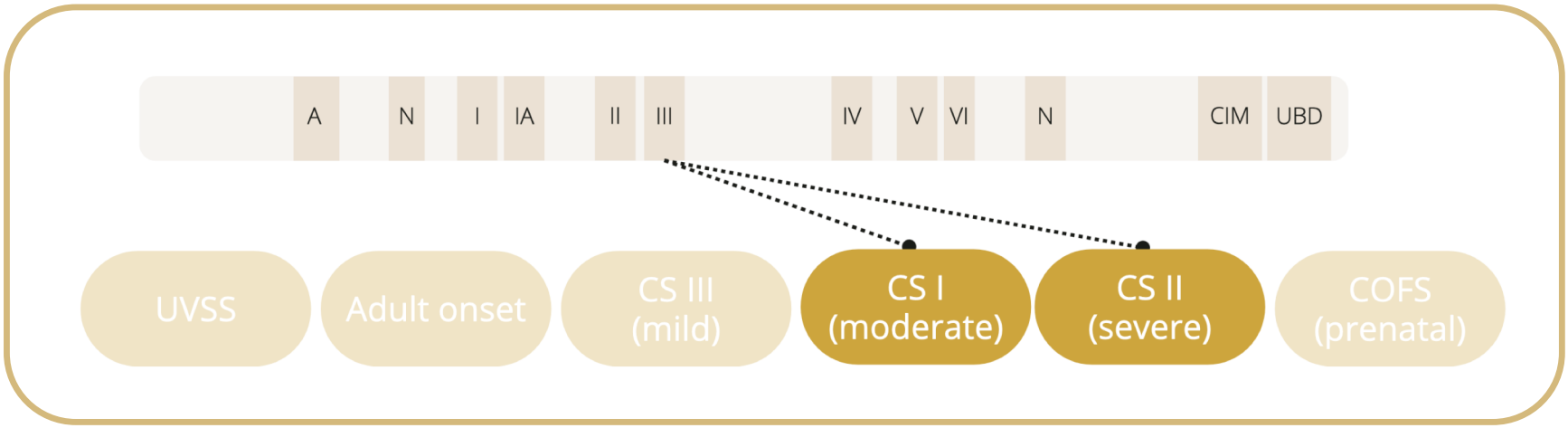
- Large clinical heterogeneity



- CSB homozygous mutations



The same homozygous mutation may results in different CS forms

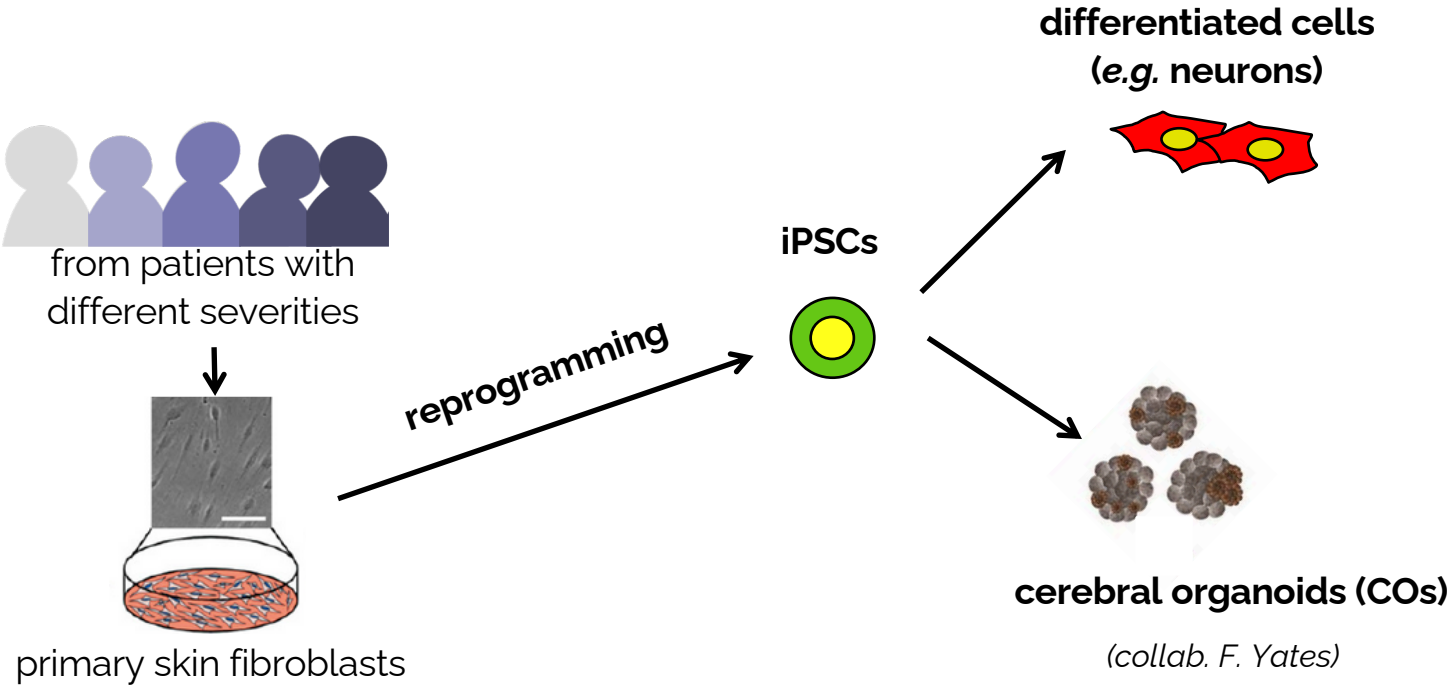


Cockayne syndrome

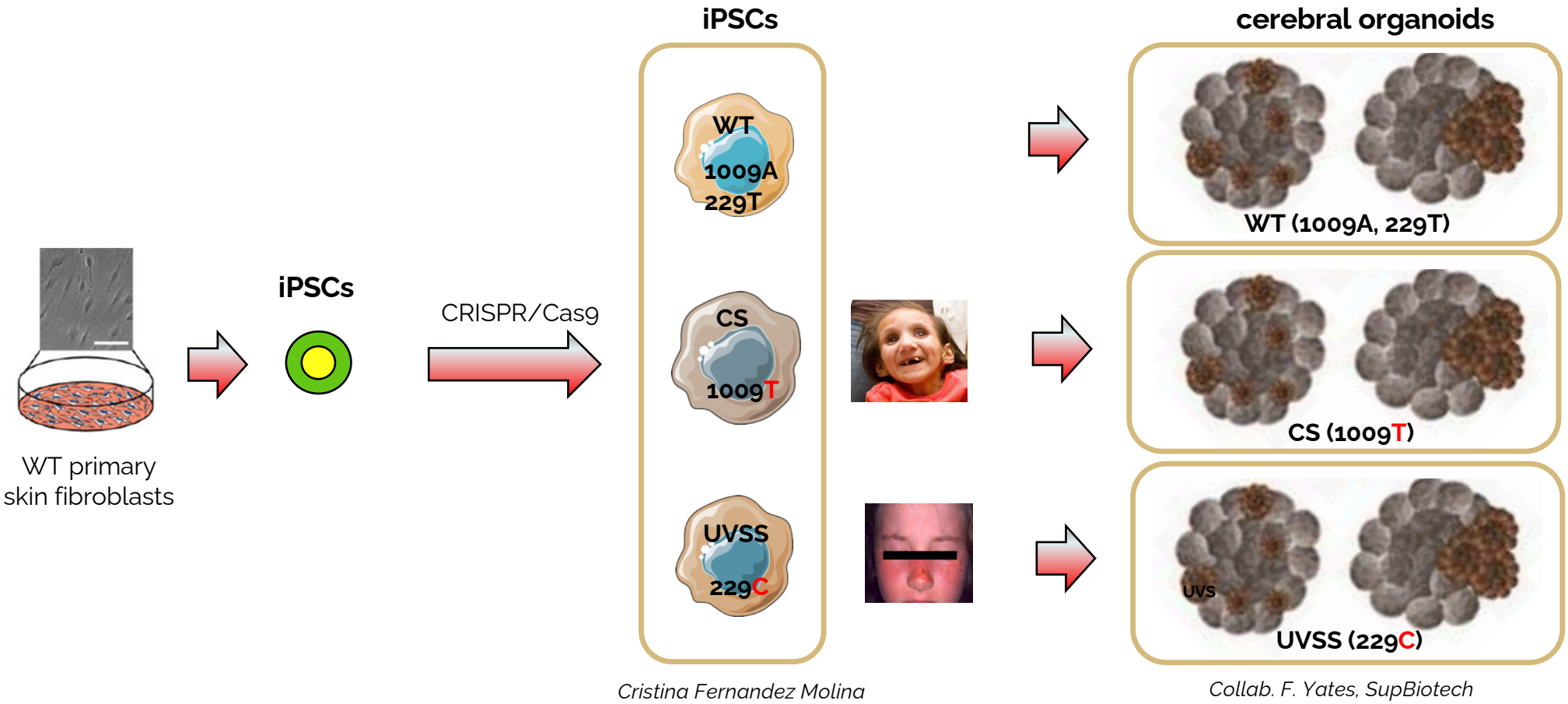
- Animal models poorly recapitulate the disease
- Large clinical heterogeneity (reason unknown)

Need to generate patient-derived cellular models

Patient-derived iPSCs and cerebral organoids



Isogenic iPSCs and cerebral organoids



Cristina Fernandez Molina

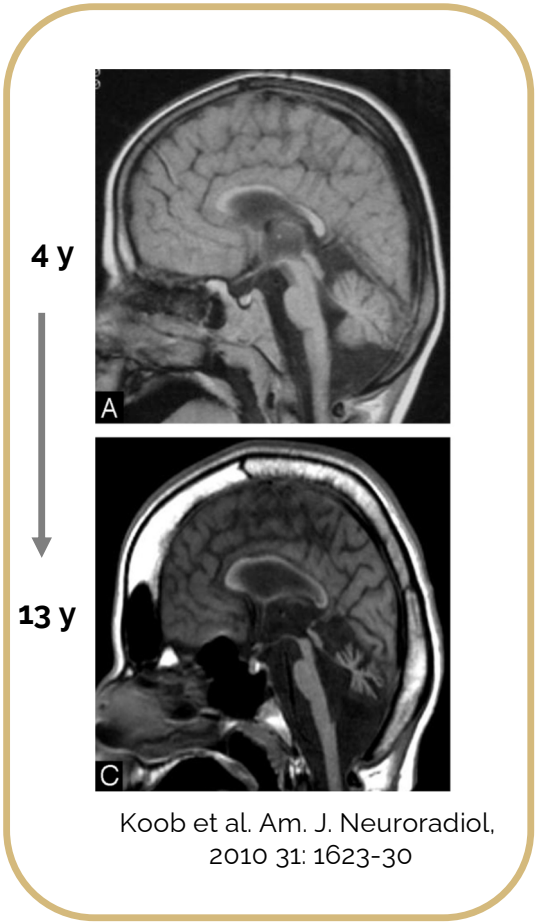
Collab. F. Yates, SupBiotech

To address the issue of the mutation/genetic background in the severity of the phenotype

Neurodegenerative conditions in Cockayne syndrome

CS is the only severe progeroid disease displaying neurodegeneration

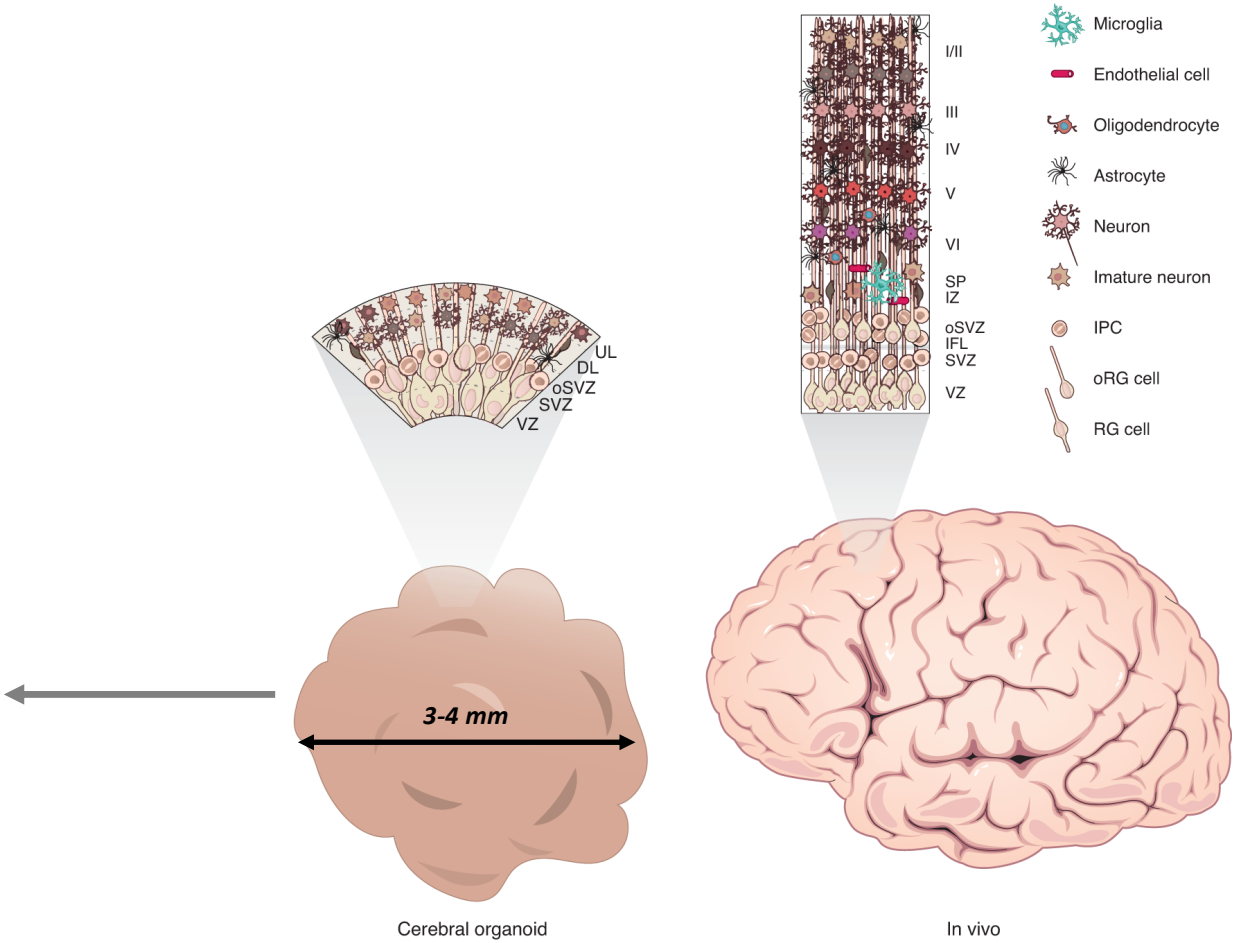
- **Microcephaly**
- (Hydrocephalus)
- Progressivee hearing loss
- Cognitive deficit
- Spastic ataxia
- Pigmentary retinopathy
- Optic atrophy
- **Hypomyelination**
- **Calcifications**
  (putamen/cortex/dentate nuclei)
- **Severe progressive brain atrophy**
  (supratentorial white matter/cerebellum/ corpus callosum/brain stem)



Koob et al. Am. J. Neuroradiol, 2010 31: 1623-30

Cerebral organoids (COs)

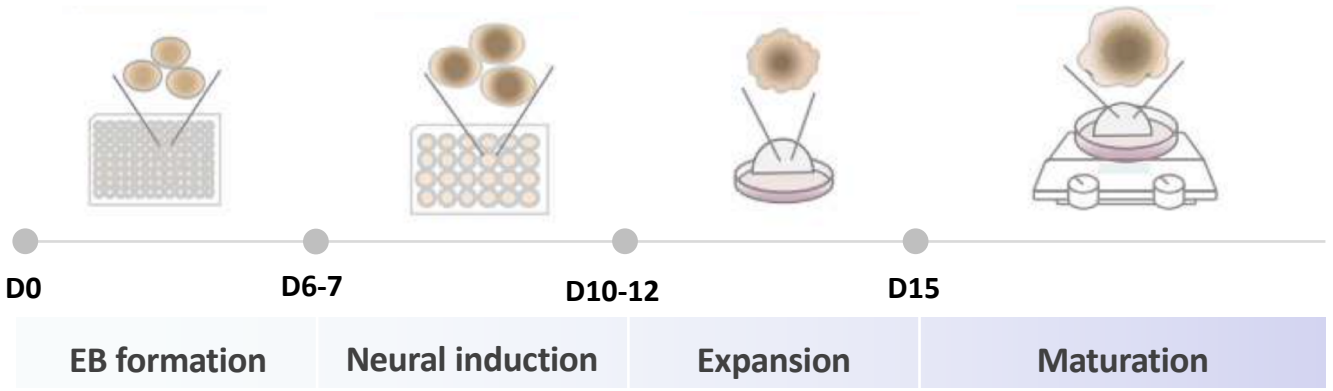
- **Self-organized 3D *in vitro* embryonic structures**
- **Long-term culture (months)**
  → longitudinal studies
  → oxidative stress
- **Mechanistic (molecular) studies**
- **Drug testing**



Modified from Chiaradia & Lancaster (Nature Neurosciences 2020, 23: 1496)

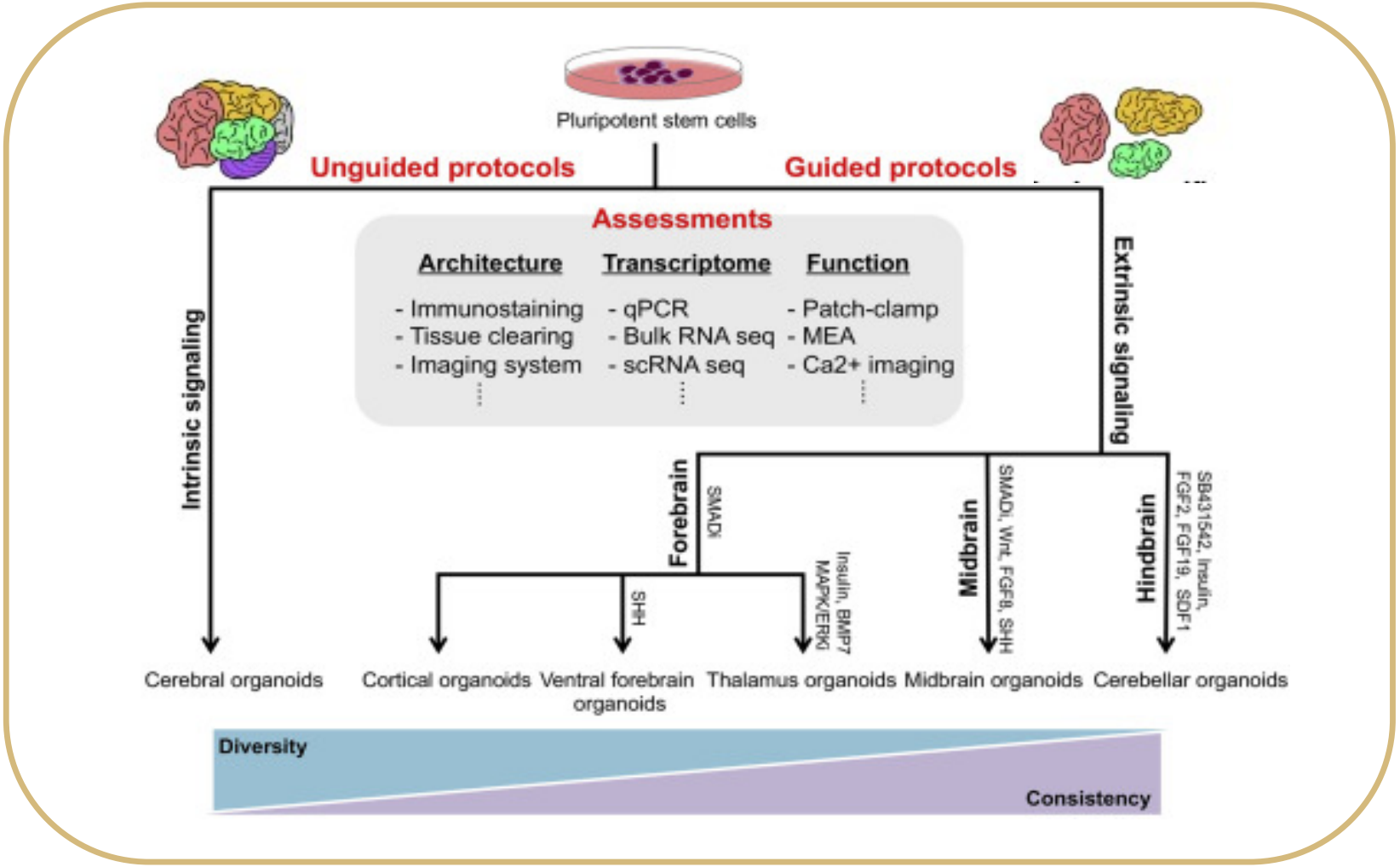
Procedure whole-brain organoids

UNGUIDED

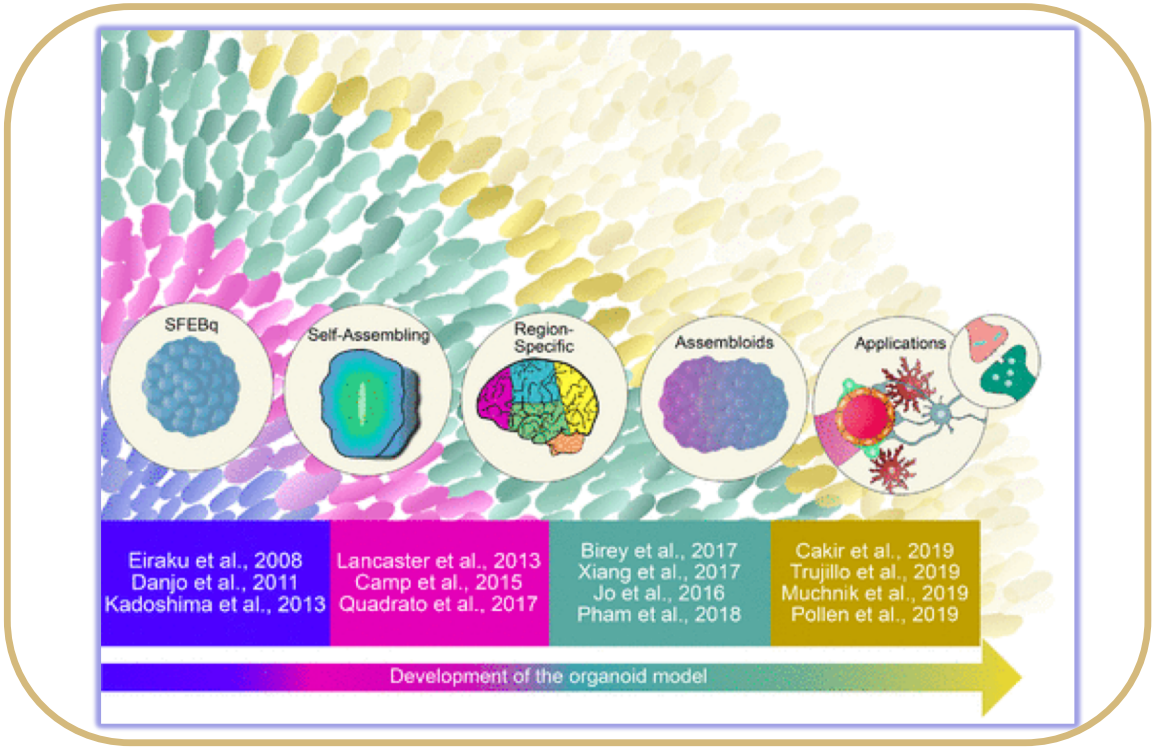


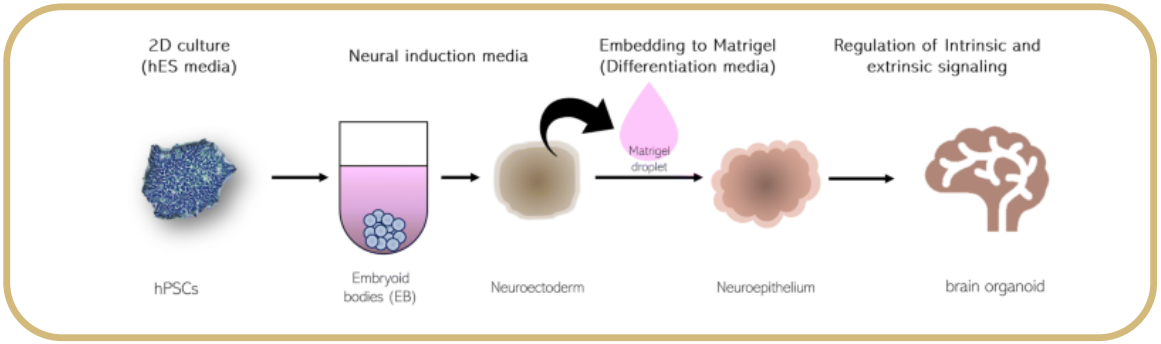
Adapted from Lancaster et al, 2013

Multiple methods and types of cerebral organoids

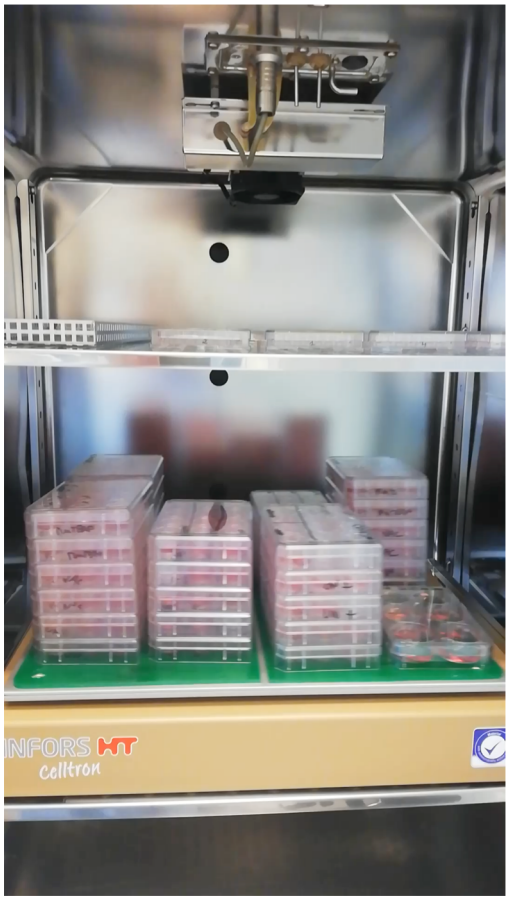


Multiple methods and types of cerebral organoids

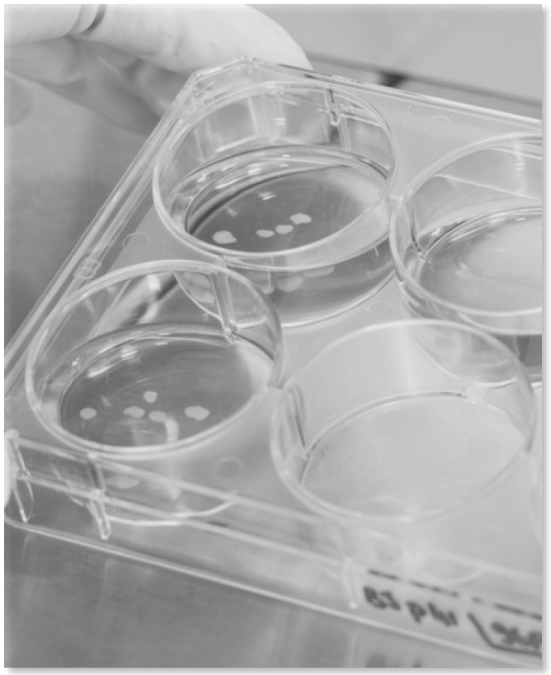




Cerebral organoids culture



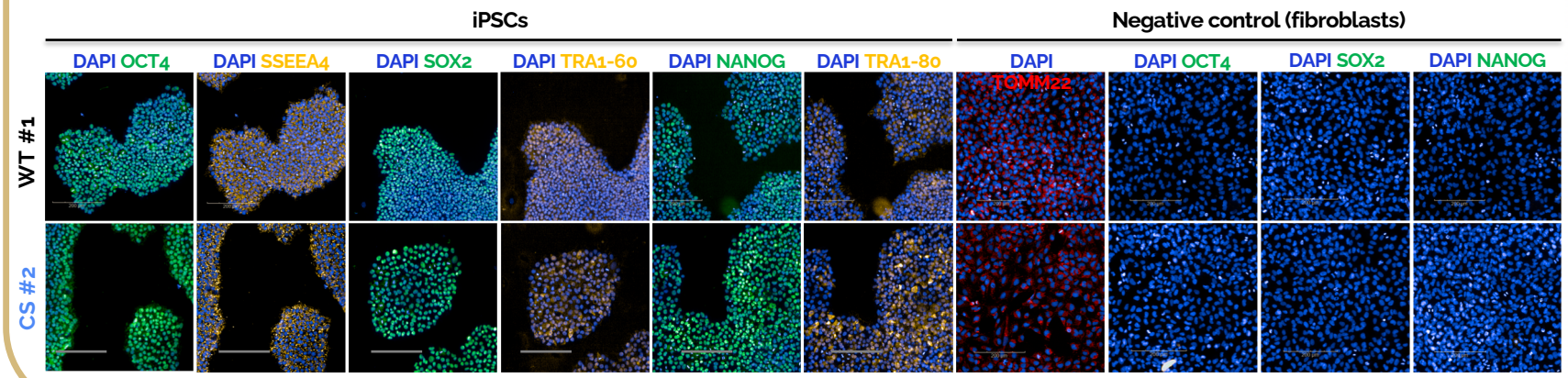
>1000 organoids in culture

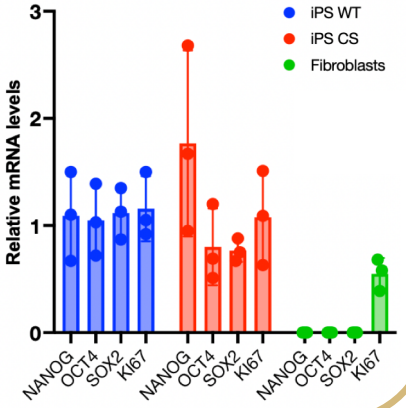


Chiara Cimmaruta

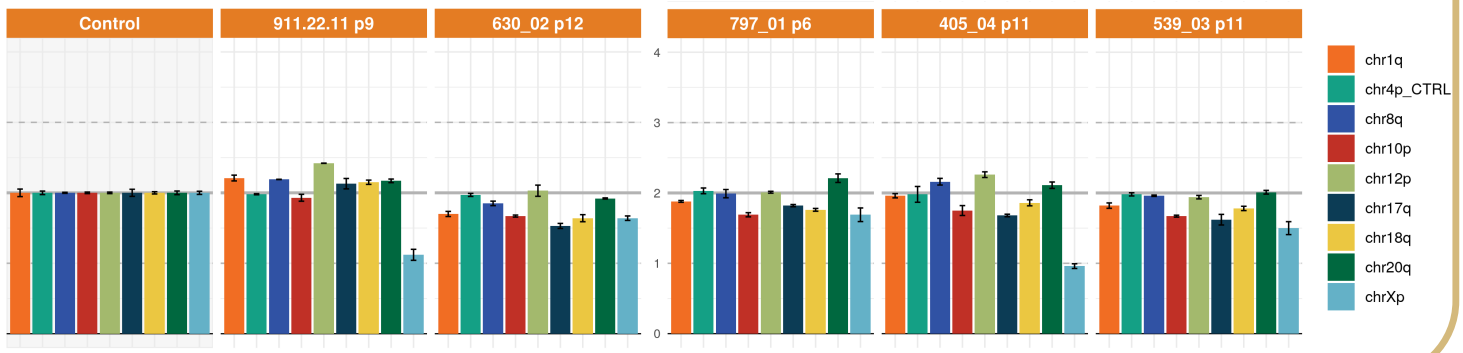
iPSCs quality control

- **Pluripotency test by IF and qPCR**





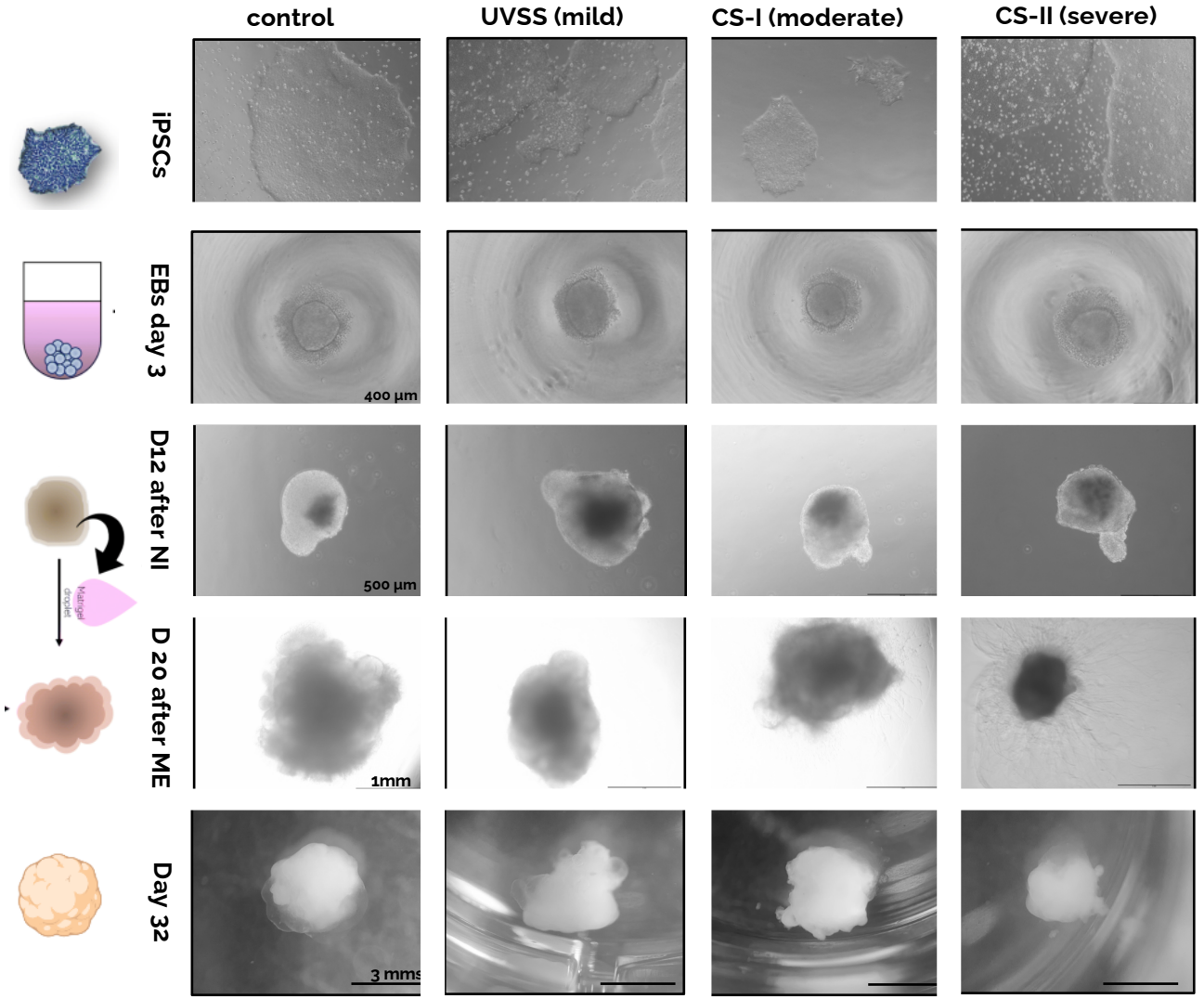
- **Karyotype analysis**



- 9 cell lines
  - 4 WT
  - 3 CS-II
  - 1 CS-I
  - 1 UVSS
- Multiple clones
- Indipendent reprogramming

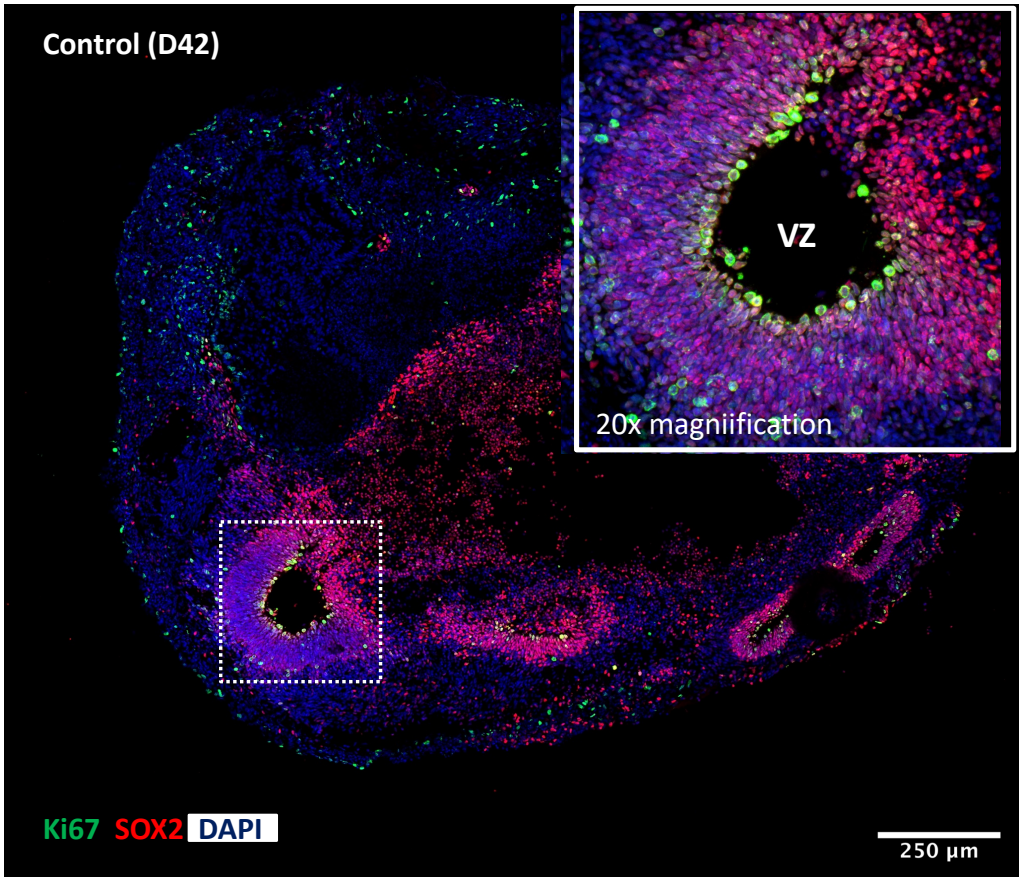
Tara Fournier and Chiara Cimmaruta

Generation of whole-brain cerebral organoids



Chiara Cimmaruta

Progenitor cells and neural rosette-like structures in COs

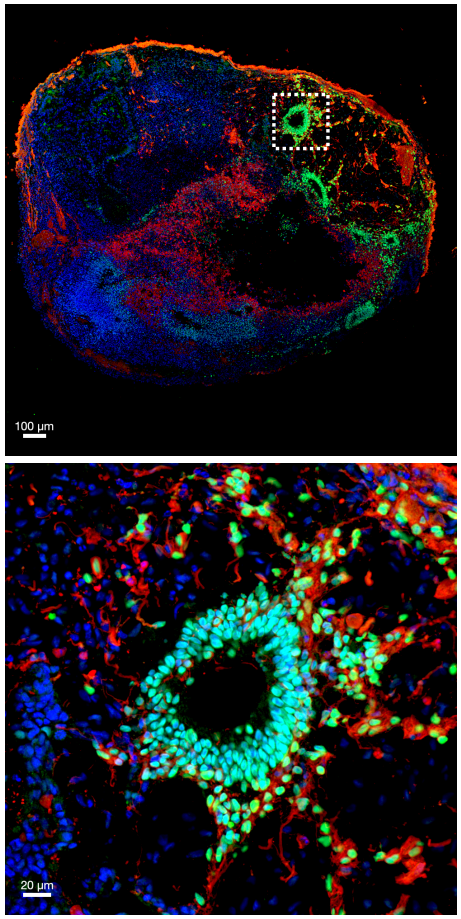


VZ, ventricular zone

Tara Fournier

Neural differentiation in cerebral organoids

Control (BJ) D42



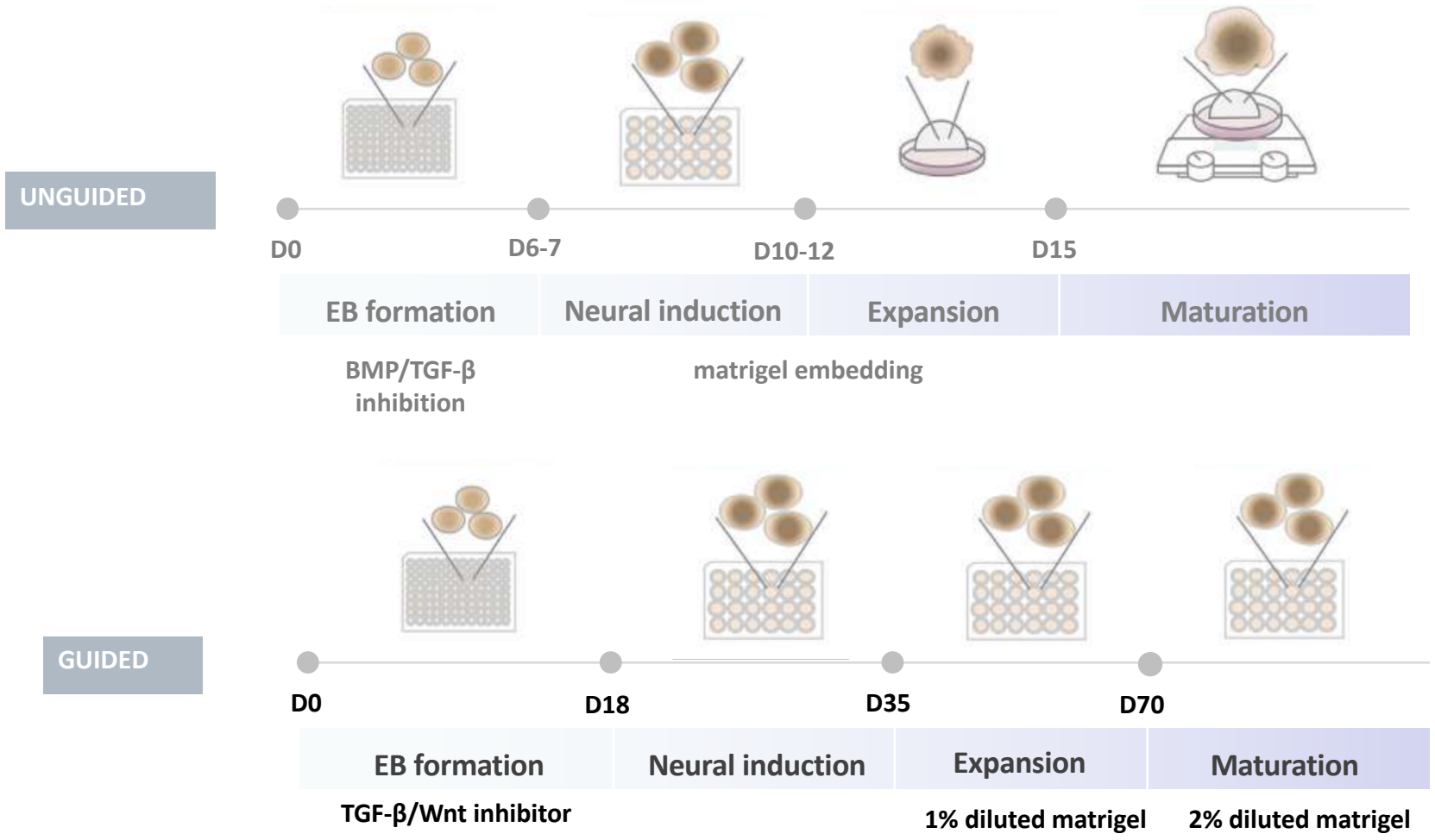
20x magnification

PAX6 TUJ1 DAPI

Altered neural differentiation in CS COs (not shown)

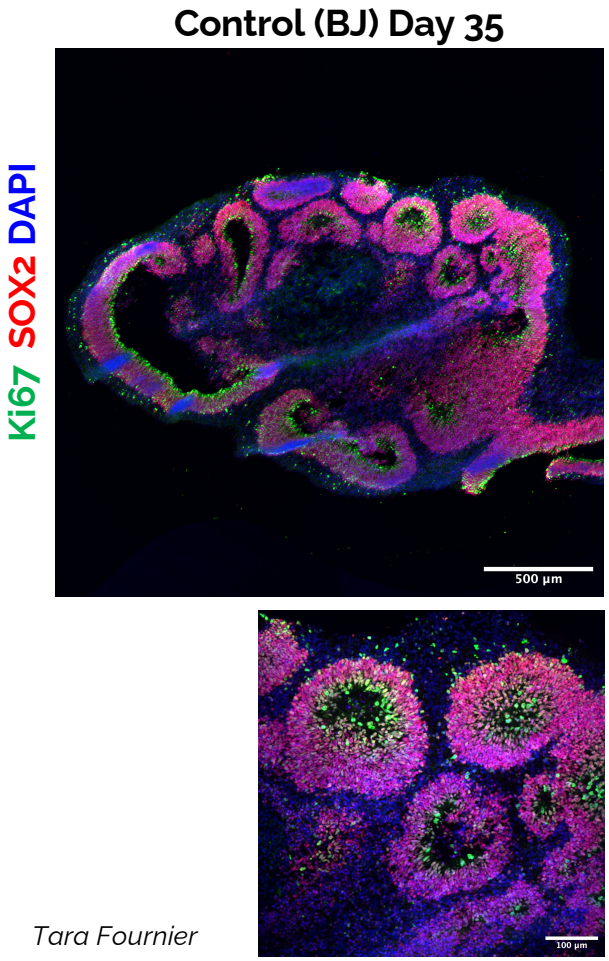
Tara Fournier

Procedure whole-brain vs. dorsal forebrain organoids



Adapted from Velasco et al, 2019

Neural rosettes in guided-COs



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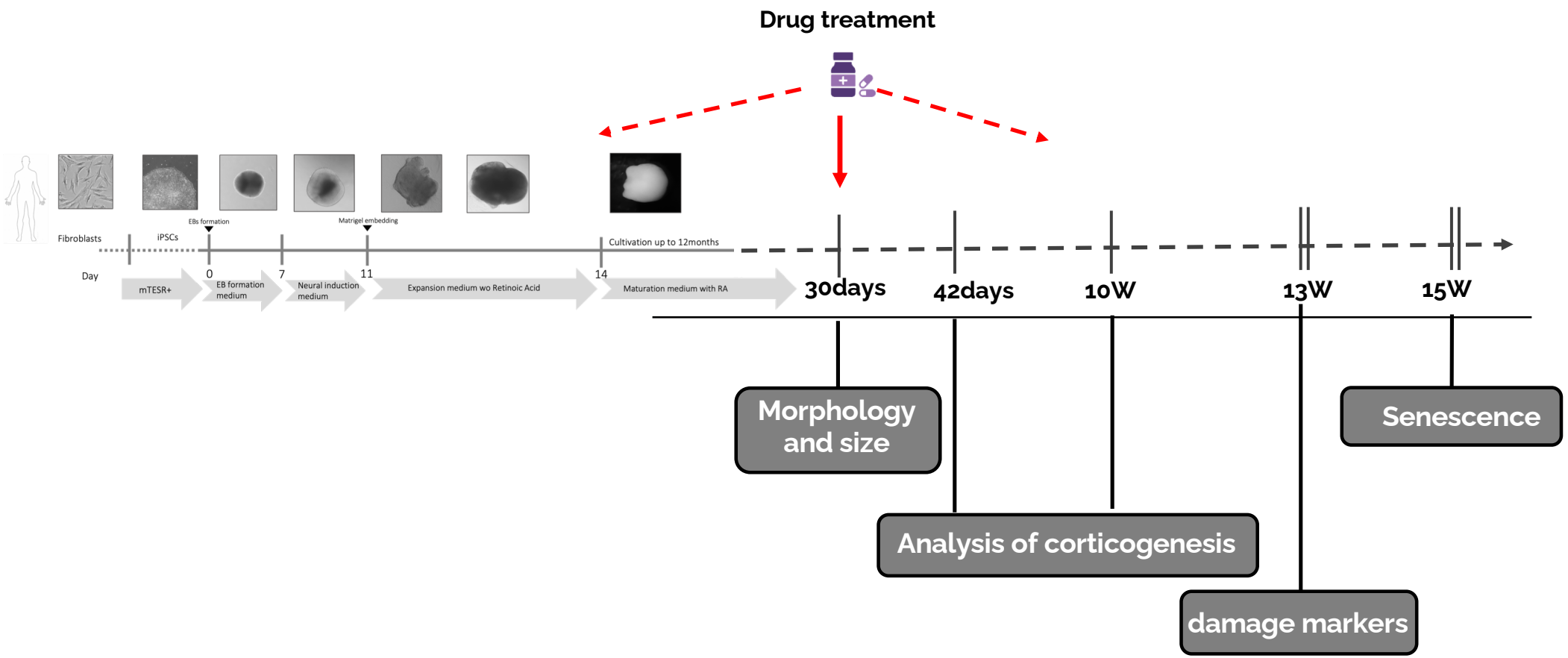
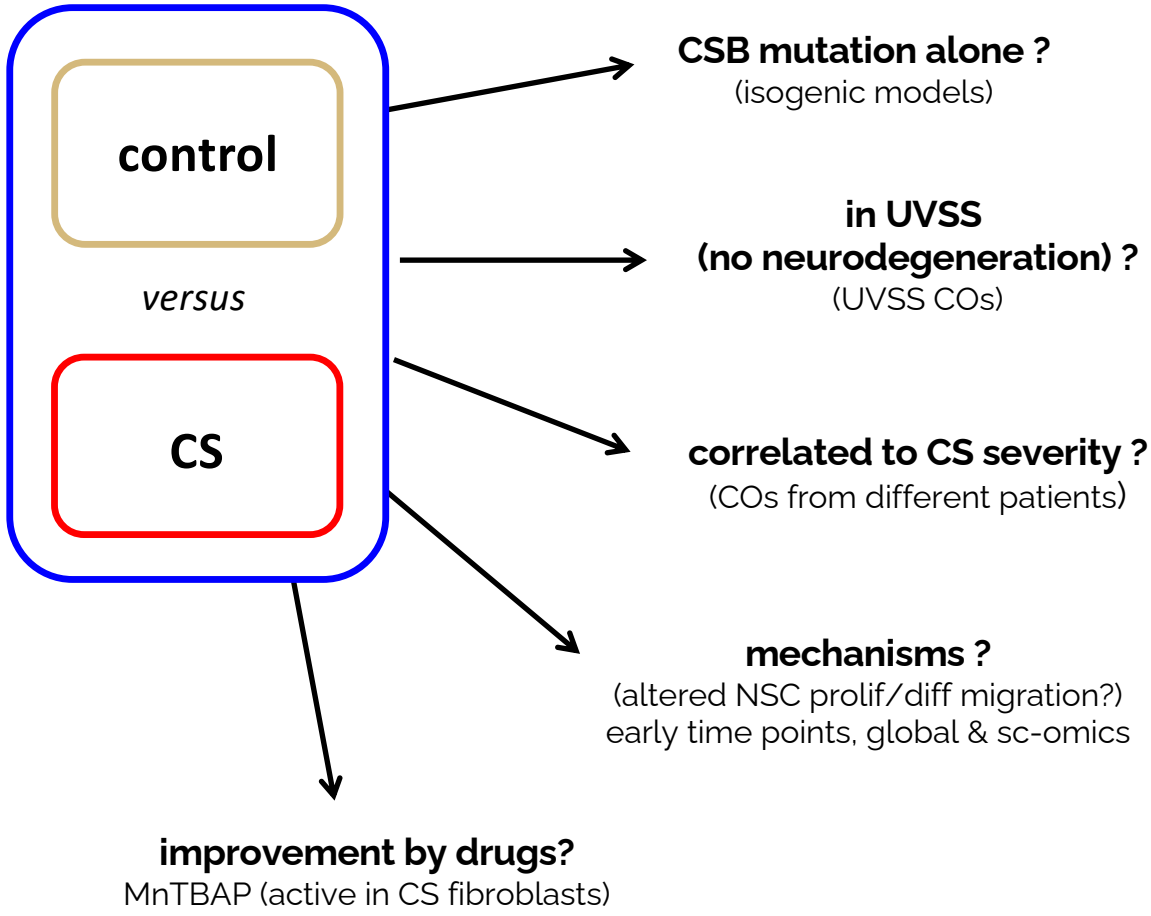
- Disrupted organization of neural rosettes in CS guided-COs
- Altered neurogenenesis in CS guided-COs

(not shown)

Pipeline to perform drug tests in cerebral orgnoids

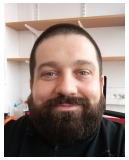


CS cerebral organoids: a functional readout



Acknowledgements

C. Crochemore (post-doc)



B. Montagne (technician)

T. Fournier (engineer/PhD)

C. Cimmaruta (post-doc)

to recruit (stud/post-doc)

former team member

C. Fernandez-Molina

Collaborations

F. Yates-F *(iPSC & organoids)*
L. Bally-Cuif, *IP (neurodevlopment)*
A. Sarasin-F *(CS expert)*
V. Laugel-F *(CS clinician)*

M. Berneburg-D (*metabolism, CS clinician)*
C. Franceschi-I (*DNAm/ ageing*)
S. Horvath-US (*methylation clock*)

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