TreeFrog therapeutics

Cell therapy for all

Founded in 2018

Co-founded by biophysicist Kévin Alessandri & stem cell biologist Maxime Feyeux.
CEO Frédéric Desdouits appointed in 2021

100+ froggies

Blending biophysicists, stem cell biologists & bioproduction engineers.
HQ in Bordeaux + hubs in Boston and Kobe

$82M raised

2021 - $75M Series B; 2019 - $7M Series A

bpifrance LGP Leonard Green & Partners Bristol Myers Squibb

Building a global footprint in cell therapy

Two sites in Bordeaux, France

Clean rooms & analytics

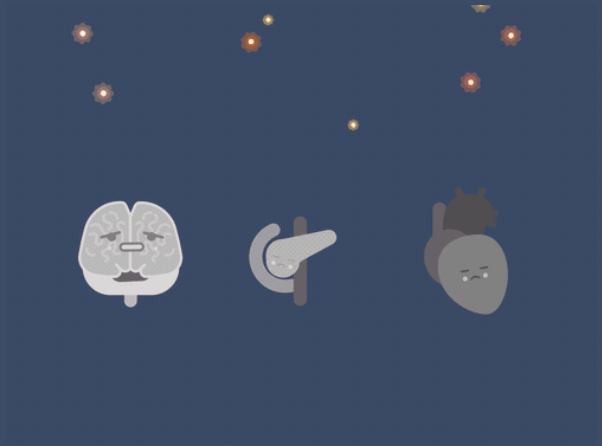
Chip manufacturing & administration

Two international hubs to drive partnership opportunities in key markets

LabCentral 238, Kendall Square, Cambridge

Creative Lab for Innovation in Kobe (CLIK)

Unlocking iPSC-derived cell therapies



#1 Scale-up

Mass-produce billions of cells at low cost to serve thousands to millions of patients

#2 Quality

Secure cell quality at scale to ensure the safety of iPSC-derived cell products

#3 Clinic & Access

Improve access to treatments by lowering costs, providing new graft format with high efficacy

#1 Scale-up

Expected Benefit from iPSCs - Scaling up

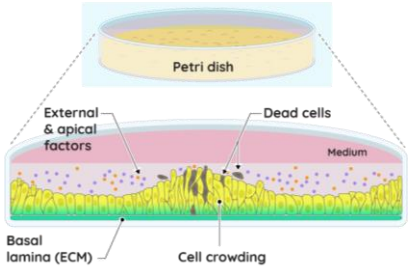
Autologous	Allogenic	iPSCs
1 patient = 1 batch **Patients own cells** **BUT** Patients own cells Limited material available for testing and validation No treatment if manufacturing failure Delay in administration	**1 donor or X donors = 1 batch = 1 to 50 - 100 patients** **Off the shelf availability** **Material available for testing and validation** **BUT** Batch to batch comparability may be challenging Donor/Patient compatibility* *hypo-immune cells approach feasible	**Banking strategy:** **1 MCB = hundreds of vials, and by establishing WCB, you can in theory treat an unlimited number of patients.** **Off the shelf availability** **Material available for testing and validation** **BUT** Donor/Patient compatibility* hPSCs specific challenges (see after) *hypo-immune cells approach feasible

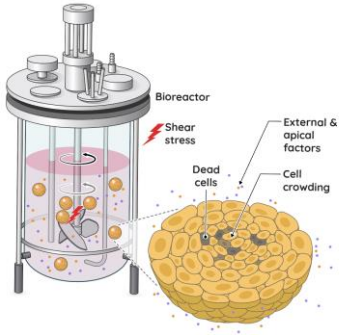
C-Stem™ addresses bottlenecks for human PSC growth

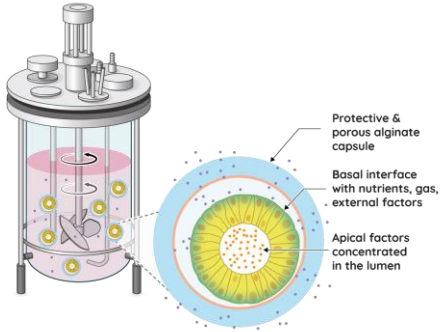
outperforming 2D and 3D approaches while maintaining *in vivo*-like cell quality & functionality

Current approaches

TreeFrog Therapeutics







	2D COLONIES	AGGREGATES IN BIOREACTORS	C-STEM™ IN BIOREACTORS
Yield (per week)	**30- to 100-fold**	**10- to 40-fold**	**100- to 300-fold**
Genomic risk	**High** Overcrowding and high mortality increasing selective pressure	**Critical** Overcrowding and high cell death (necrotic cells and impeller-induced cell death) driving selective pressure	**Contained** In vivo-like growth with low mortality; low selective pressure for oncogenic mutations
Scalability	**Scale-out only** Labor-intensive, batch-to-batch variability; high QC costs	**Scale-up limited to 3L** Due to impeller-induced shear stress damaging cells	**No limitation in bioreactor size** Cells protected by the capsule; cell culture parameters preserved across scales

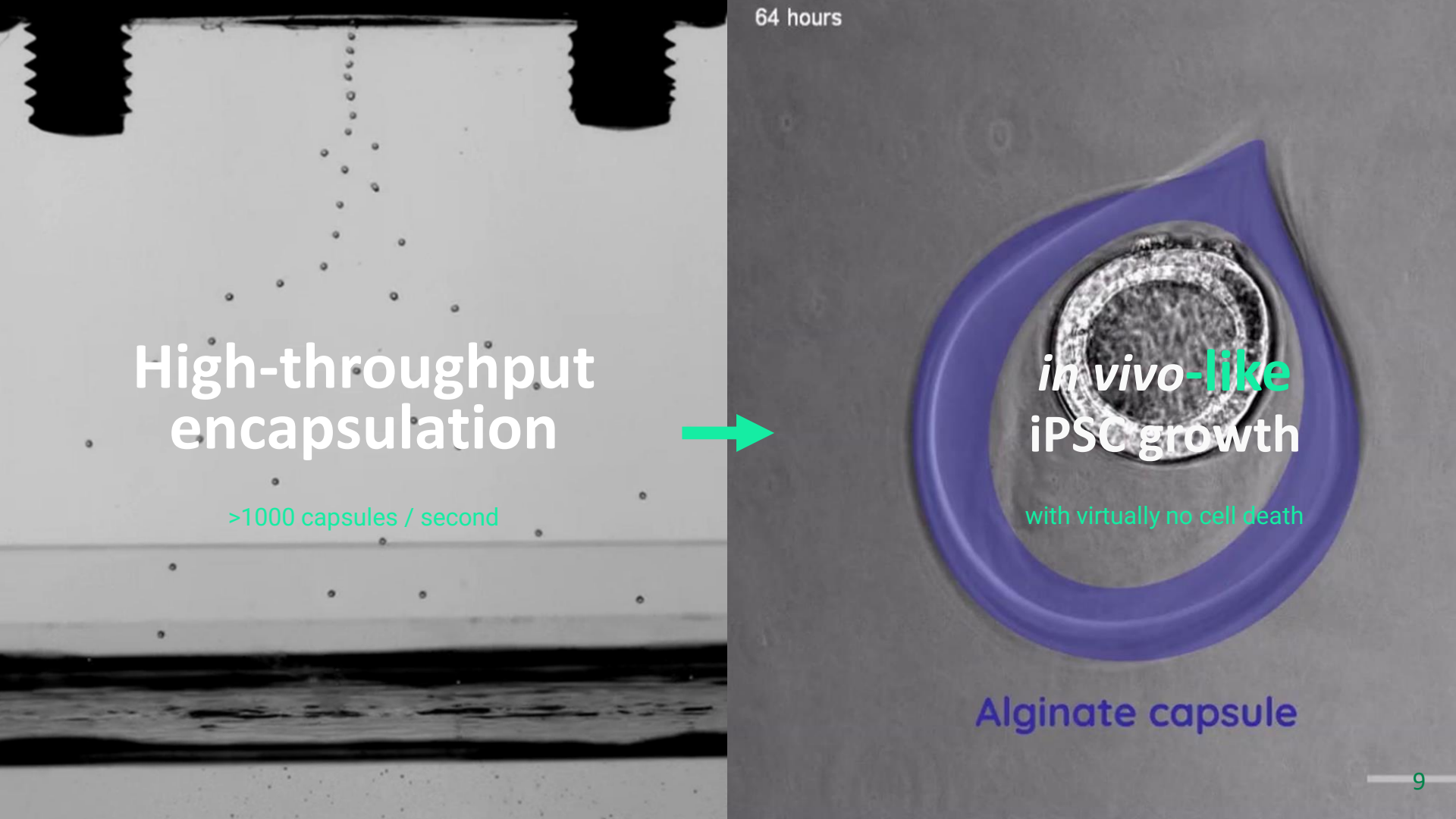
Source: TreeFrog White paper "The Lumenized Rosette, mimicking the in vivo micro-environment of human pluripotent stem cells", 2023

High-throughput encapsulation

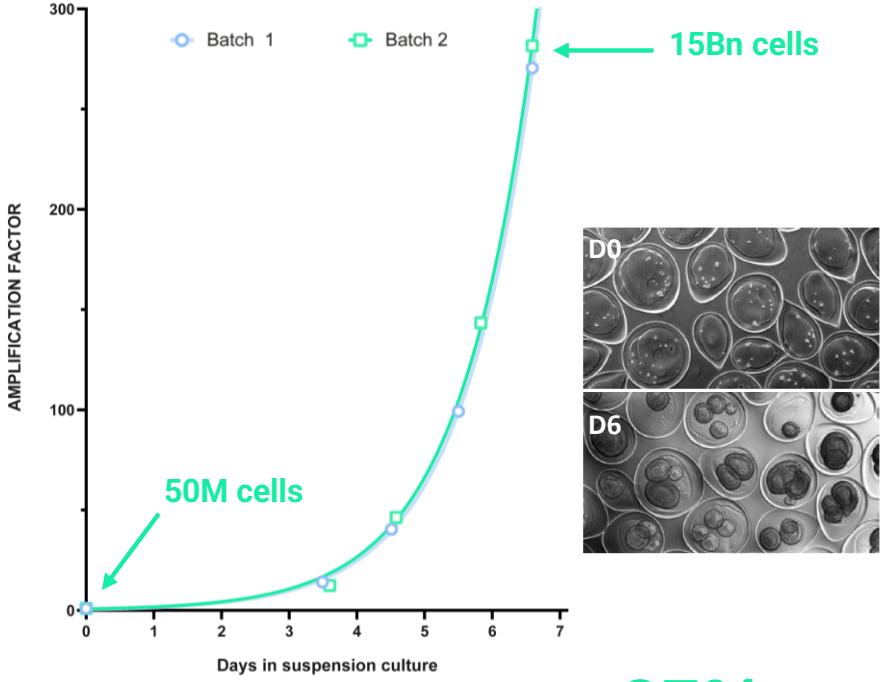
>1000 capsules / second

GMP encapsulation device

Automated, closed, single-use environment


64 hours
High-throughput encapsulation
>1000 capsules / second
in vivo-like iPSC growth
with virtually no cell death
Alginate capsule

Highly reproducible exponential iPSC expansion in 10L bioreactors (2021)



>98%

High viability
(dead cells not rinsed away as in 2D)

>97%

Robust pluripotency across batches
95.2% OCT4 / 99.5% SOX2 / 98.3% NANOG

See Cohen *et al.*, Biomaterials, 2023

Unprecedented iPS amplification in 10L bioreactors*

276x

in 6.59 days

1 batch, 1 week = 15Bn hiPSCs

*Best performance previously reported in literature:37-fold in 6 days in 10L bioreactor (Huang *et al* 2020), with significant drop in stemness

Cell Therapy for ALL

With the scale, cost reduction can be obtained by:

- **Savings on materials and consumables**
- **Batch size allowing to treat hundreds of patients**
- **Automation of production**
- **Access to treatments despite limited starting material**

Benchmarking Cell Culture Systems for iPSCs

Continuous culture over 28 days

2D culture

P0 P1 P2 P3 P4 P5 P6

Aggregates

P0 P1 P2 P3 P4 P5

C-Stem™

P0 P1 P2 P3

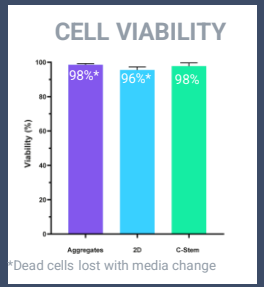
GENOMIC BENCHMARK

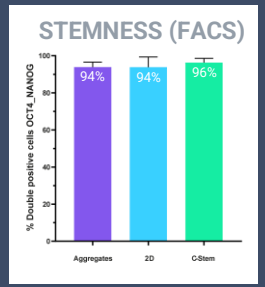
2D vs Aggregates vs C-Stem™

C-Stem: fastest growth, with reduced passages over 28 days

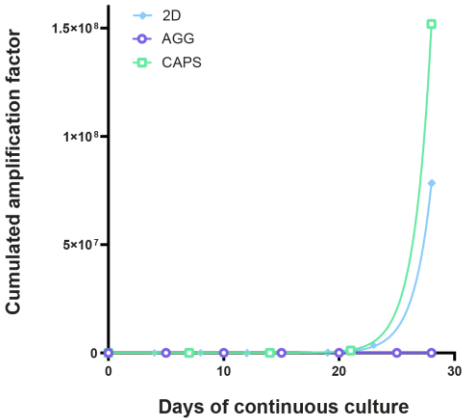
hiPSC quality : matching standard systems

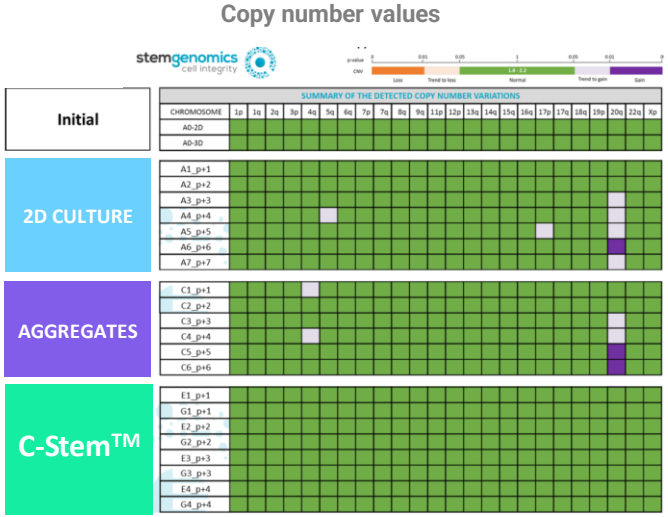
(in which dead cells are rinsed away)





Controlling pre-oncogenic 20q11 mutation throughout large-scale expansion

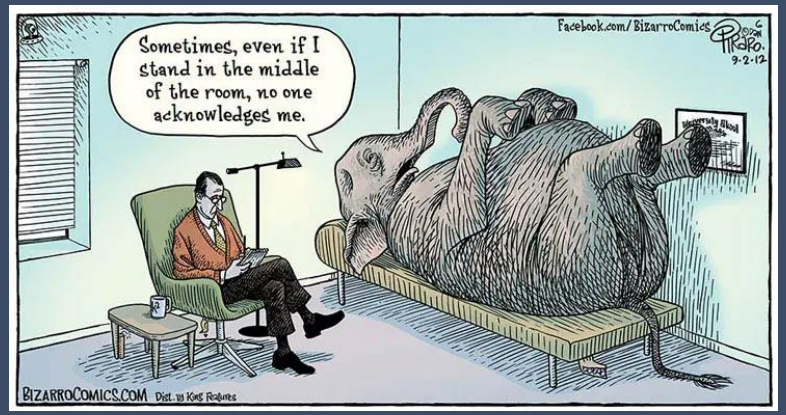




Starting material: hiPS cell line containing undetectable levels* of cells with a 20q11. 21 duplication. Head-to-head comparison of 2D, aggregates and C-Stem™ formats. While providing the best amplification performance, C-Stem™ is capable of controlling copy number variations throughout prolonged culture.

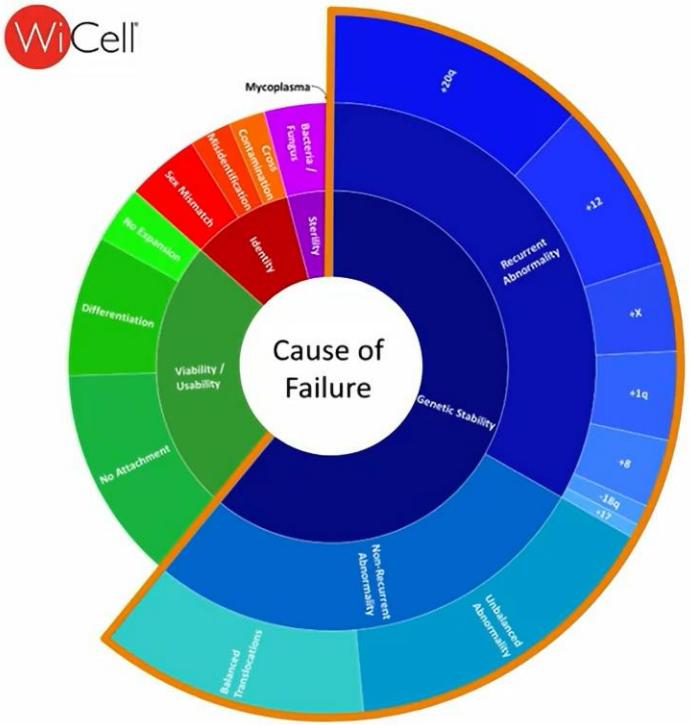
*below the limit of detection (LOD)

FOCUS ON: iPSC Cell Lines Requirements



- Genetic Stability is an ongoing issue issue in the pluripotent stem cell cell field
- Not All Cell Lines are of the same quality
- There is currently no standard approach on genes to be monitored for cell line selection

How good are iPSC Lines ?



Study of 839 hPSC lines, 285 failed testing (34%)

61% - Genetic Stability (G-banding)
- Half of which were recurrent abnormalities

25% - Viability/Usability
- No attachment, expansion or excessive differentiation

10% - Identity (Short Tandem Repeats)
- Identity mismatch, sex mismatch or mixed cell population

4% - Sterility
- Non-sterile or mycoplasma positive

D. Felkner., J. Brehm, E. McIntire, S. Minter, A. Paguirigan, K. Remondini, S. Taapken & T.E. Ludwig. "Human pluripotent stem cell quality: A scientific wake-up call." Poster presented at the ISSCR Annual Meeting; June 2019; Los Angeles, California.

What we know:

- Karyotypic abnormalities are acquired throughout culture in a spontaneous and nonrandom manner (K 1, 12, 17, 20, and X)
- It provides competitive advantage to iPSCs through different mechanisms (attachment after seeding, faster cell cycle times, reduced death, reduced differentiation…)
- They are similar to karyotypic abnormalities observed in human cancers.

Are iPSC Cell Line good enough for clinic?

Consensus = nogo	In discussion	Unknown
Cell Lines with complete chromosome duplications or deletions cannot be used in clinic COSMIC list, PMDA List (2013) TP53, BCOR	What to do with SNV, CNV? ISSCR recommendations on Common recurrent genetic changes in hPSCs, some of their phenotypic consequences and suitable methods for their detection	What if below the level of detection? CNV Treesholds? Number? Size? Kinetics? Acceptability if no observation in NC and tumourogenicity studies? How does RBA support Clinical use

What's Next? if we want iPSCs' potential to become become a reality

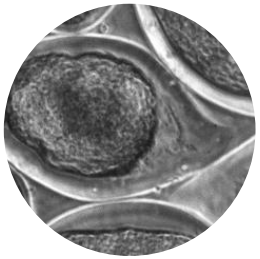
- **Industry:**
  - **Shared responsability to look at the issue:**
    - Cell Line suppliers
    - Drug Developpers
  - **Support the establishment of standards by generating data**
  - **Be transparent to support RBA if applicable.**

- **Regulators / Pharmacopeia:**
  - **Need a stakeholder workshop / White paper to pave the way for Guidance on IPSC Cell line quality requirements, choice is made very early in development (go/nogo)**
  - **Standards on oncogenic panel testing will harmonize practices, improve safety and allow for easier identification of the suitable materials from suppliers.**


#3 Impact on Clinic & Access
CONFIDENTIAL PRESENTATION

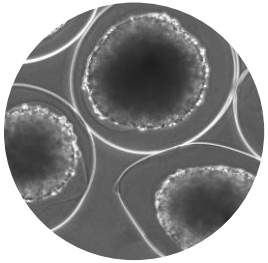
C-Stem™: unlocking cell therapy potential

hiPSC-derived microtissues



Neural
Lead program (Parkinson's)

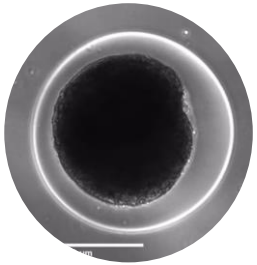
in house



Liver
9 months from concept to IP

in house

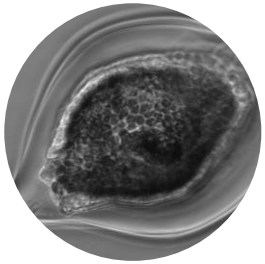
partnering



Cardiac
6 months from concept to IP

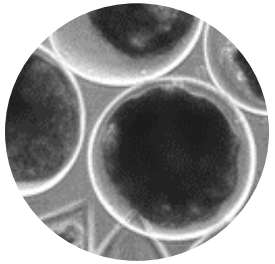
in house

partnering



RPE
3 months from concept to IP

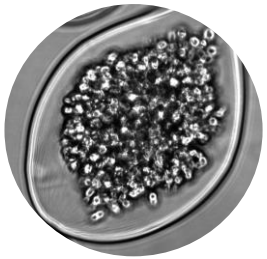
in house



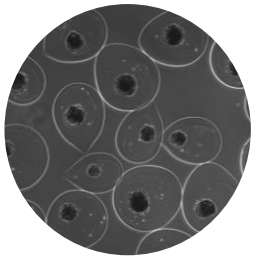
CD34+
6 months to launch *in vivo* PoC

partnering

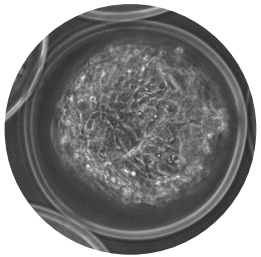
Donor-derived microtissues



T cells
3 months from concept to IP



NK cells
On-going PoC

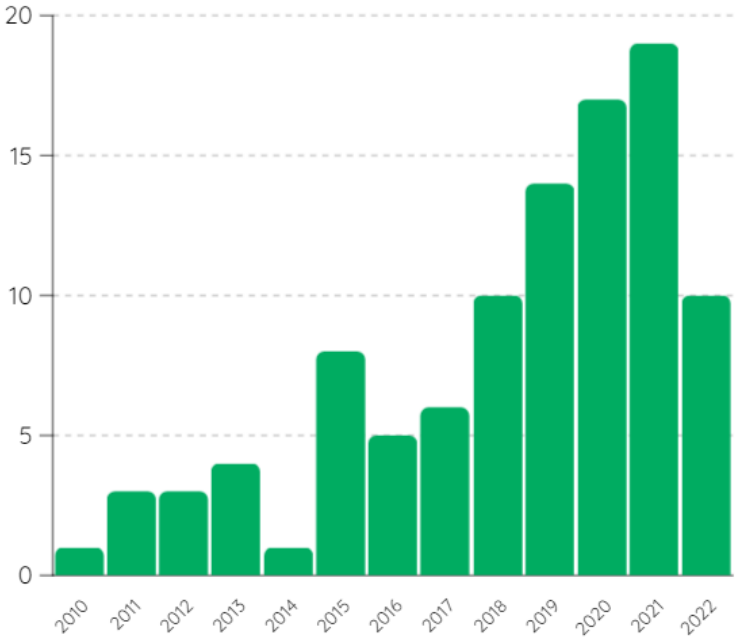


MSCs
6 months for PoC study

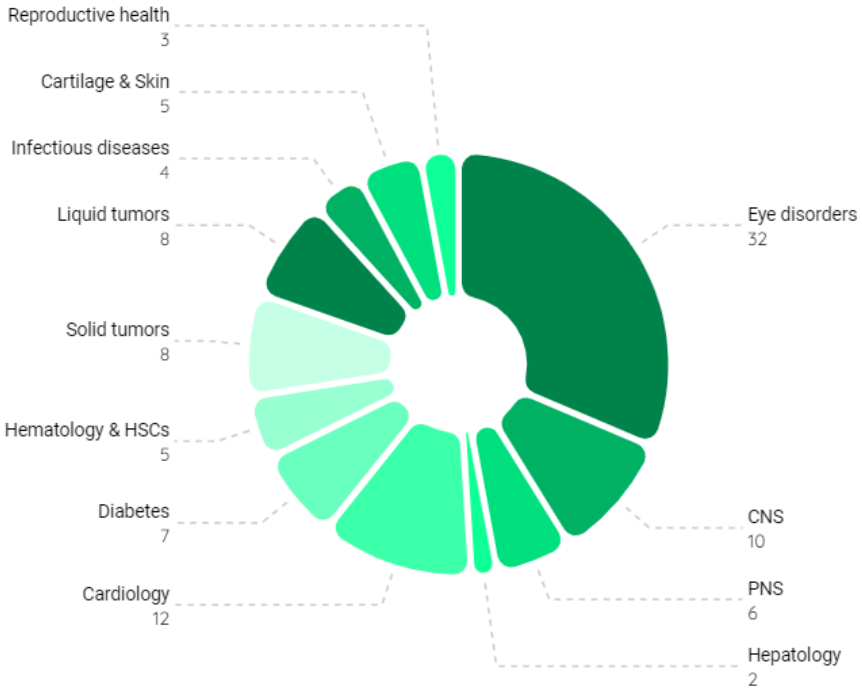
> 100 hundred trials based on pluripotent stem cells

yet, no significant clinical progress beyond Phase I/II

Number of clinical trials based on pluripotent stem cells launched per year



Number of clinical trials based on pluripotent stem cells per disease area



Sources: TreeFrog's internal database about PSC-based trials : https://airtable.com/shrzzoHVEgulGmry6

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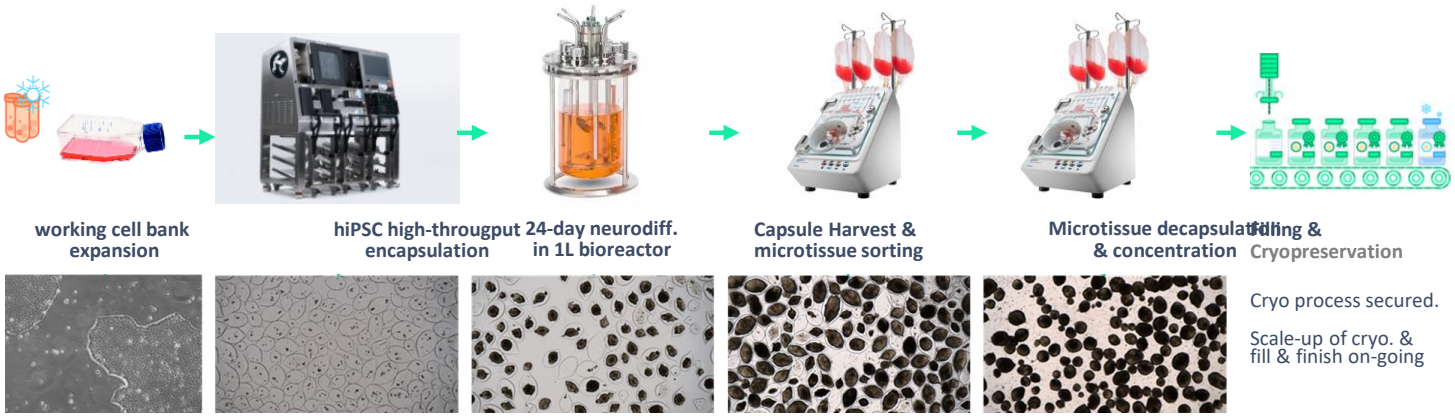
C-Stem™ -based GMP Manufacturing Process & QC

Bringing automation, 800 doses in 1L bioreactor



42 days

In-process QC

- Viability
- Identity
- Morphology

Batch release QC

- Quantity/Viability
- Purity
- Impurities
- Potency (dopa release)
- Karyotyping

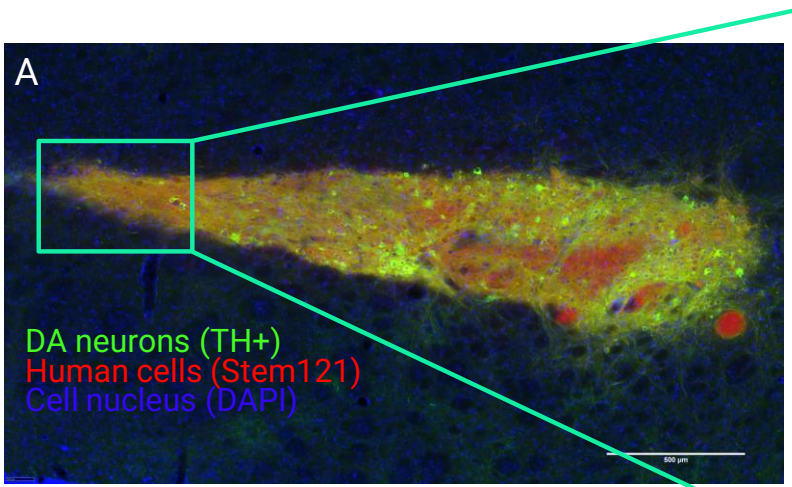
Additionnal QC

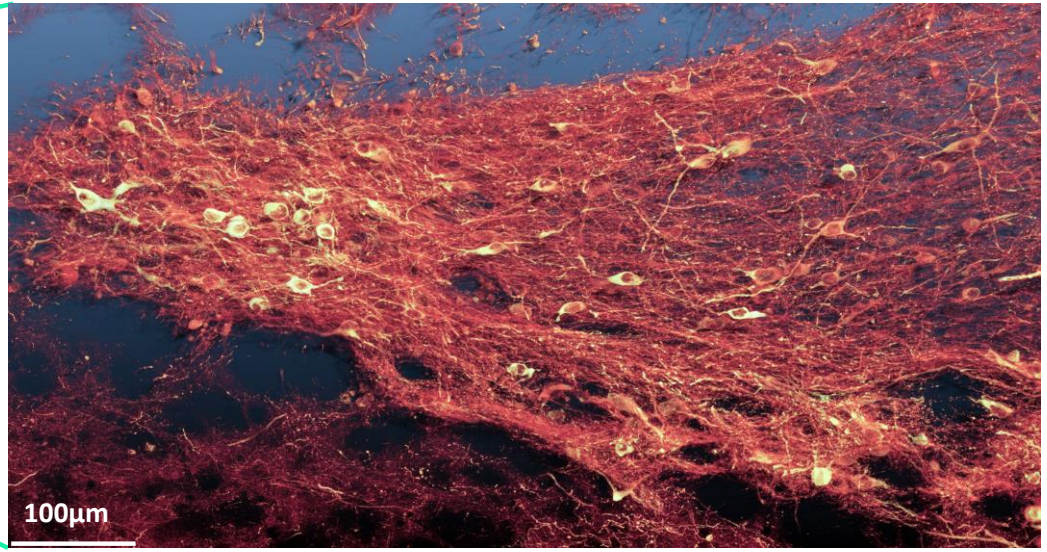
- Genomic integrity (CNV)
- Single-cell characterization

Neural microtissue engrafts, dopaminergic neuron projections observed

The implanted human microtissues survived, thrived in the lesioned hemisphere of the rat brain post implantation

TH positive dopaminergic neurons cell bodies detected in the graft area

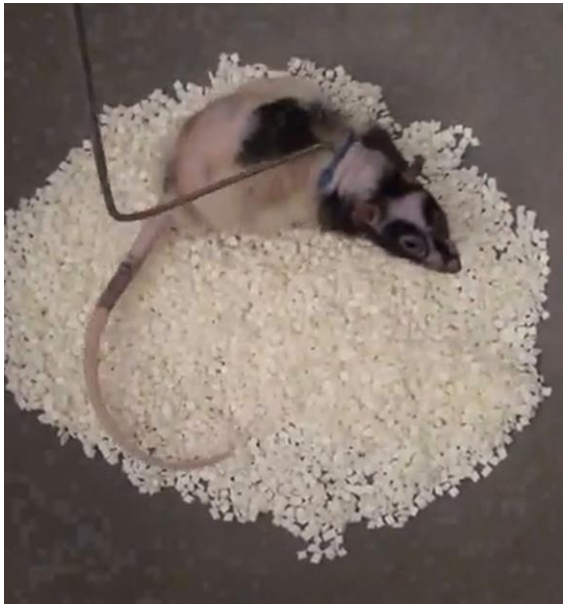




Cryopreserved microtissues transplanted (high dose) within the dorsal striatum after a 5 months ± 10 days of *in vivo* experiment period
(A) Overview of the whole grafted area and (B) observations of the TH+ cells projection within the host tissue.

Full motor function recovery: 8 weeks

Hemiparkinsonian pre-graft (rat #13)



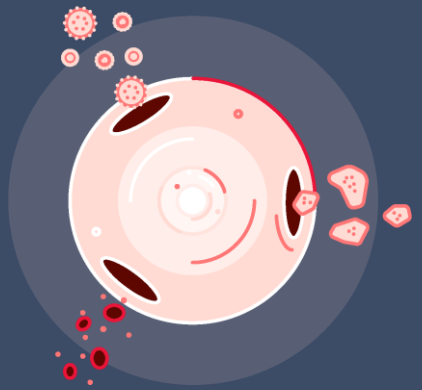
8 weeks after C-Stem™ neuron implantation (rat #13)

Hemiparkinsonian pre-graft (rat #13)

C-stem™ platform : unlock iPSC potential

Fullfilling
the iPSC promise
From 1 good edit
to billions of cells

Meet CMC expectations
for allogeneic products Scale,
automation,
quality



Create unique
products for various indications with
unique phenotypic specs
unique 3D format

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