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

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

<table>
<thead>
<tr>
<th></th>
<th>Autologous</th>
<th>Allogenic</th>
<th>iPSCs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1 patient = 1 batch</strong></td>
<td>Patients own cells</td>
<td>1 donor or X donors = 1 batch = 1 to 50 - 100 patients</td>
<td>Banking strategy: 1 MCB = hundreds of vials, and by establishing WCB, you can in theory treat an unlimited number of patients.</td>
</tr>
<tr>
<td><strong>BUT</strong></td>
<td>Patients own cells</td>
<td>Off the shelf availability</td>
<td>Off the shelf availability</td>
</tr>
<tr>
<td></td>
<td>Limited material available for testing and validation</td>
<td>Material available for testing and validation</td>
<td>Material available for testing and validation</td>
</tr>
<tr>
<td></td>
<td>No treatment if manufacturing failure</td>
<td>BUT</td>
<td>BUT</td>
</tr>
<tr>
<td></td>
<td>Delay in administration</td>
<td>Batch to batch comparability may be challenging</td>
<td>Donor/Patient compatibility*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>hPSCs specific challenges (see after)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>*hypo-immune cells approach feasible</td>
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<td>*hypo-immune cells approach feasible</td>
</tr>
</tbody>
</table>
C-Stem™ addresses bottlenecks for human PSC growth outperforming 2D and 3D approaches while maintaining *in vivo*-like cell quality & functionality

### Current approaches

<table>
<thead>
<tr>
<th>2D COLONIES</th>
<th>AGGREGATES IN BIOREACTORS</th>
<th>C-STEM™ IN BIOREACTORS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Yield (per week)</strong></td>
<td><strong>30- to 100-fold</strong></td>
<td><strong>10- to 40-fold</strong></td>
</tr>
<tr>
<td><strong>Genomic risk</strong></td>
<td>High</td>
<td>Critical</td>
</tr>
<tr>
<td>Overcrowding and high mortality increasing selective pressure</td>
<td>Overcrowding and high cell death (necrotic cells and impeller-induced cell death) driving selective pressure</td>
<td>In vivo-like growth with low mortality; low selective pressure for oncogenic mutations</td>
</tr>
<tr>
<td><strong>Scalability</strong></td>
<td>Scale-out only</td>
<td>Scale-up limited to 3L</td>
</tr>
<tr>
<td>Labor-intensive, batch-to-batch variability; high QC costs</td>
<td>Due to impeller-induced shear stress damaging cells</td>
<td>Cells protected by the capsule; cell culture parameters preserved across scales</td>
</tr>
</tbody>
</table>

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
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)

Unprecedented iPSC amplification in 10L bioreactors*

1 batch, 1 week = 15Bn hiPSCs

Unprecedented iPS amplification in 10L bioreactors*

>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

*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
#2 Quality
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
Controlling pre-oncogenic 20q11 mutation throughout large-scale expansion

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

hiPSC quality: matching standard systems (in which dead cells are rinsed away)

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 in the pluripotent stem 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

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?

<table>
<thead>
<tr>
<th>Consensus = no go</th>
<th>In discussion</th>
<th>Unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell Lines with complete chromosome duplications or deletions cannot be used in clinic</td>
<td>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</td>
<td>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</td>
</tr>
<tr>
<td>COSMIC list, PMDA List (2013) TP53, BCOR</td>
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**CONFIDENTIAL PRESENTATION**
What's Next? if we want iPSCs' potential to become a reality

**Industry:**
- Shared responsibility to look at the issue:
  - Cell Line suppliers
  - Drug Developers
- 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
C-Stem™: unlocking cell therapy potential

- hiPSC-derived microtissues
  - Neural: Lead program (Parkinson's)
  - Liver: 9 months from concept to IP
  - Cardiac: 6 months from concept to IP
  - RPE: 3 months from concept to IP
  - CD34+: 6 months to launch in vivo PoC
  - in house
  - 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
Parkinson's disease cell therapy
C-Stem™-based GMP Manufacturing Process & QC

Bringing automation, 800 doses in 1L bioreactor

- Viability
- Identity
- Morphology

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

In-process QC

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)
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