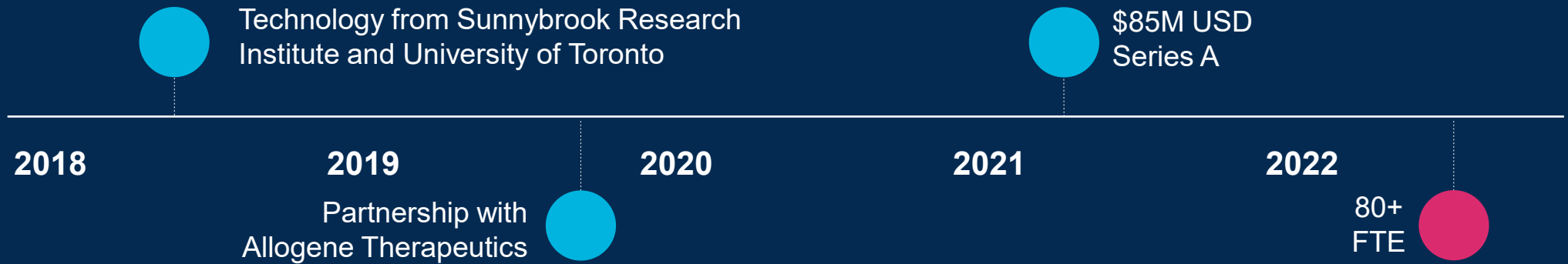


**Notch**  
THERAPEUTICS

# Enabling iPSC Manufacture to Produce Cells of High Quantity and Quality

**Liz Csaszar**  
Sr Director Manufacturing Sciences  
July 2022

# Notch is developing T cell products from renewable iPSCs



- World experts in control of Notch Signaling during cell development
- Optimizing uniformity and potency to improve iPSC-derived T cell function
- Commercially-compatible stem cell manufacturing
- Sophisticated product design

➔ End-to-end capabilities for iPSC-based therapeutics

# iPSC-based T cells can revolutionize patient outcomes

## Limitations to current CAR-T therapies

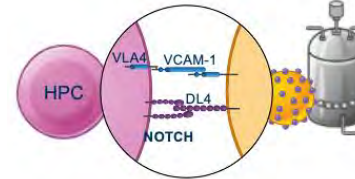
- Adult T cells are limited by the health and composition of the donor's immune system
- Unpredictable product composition
- Inaccessible to many patients
- Decentralized manufacturing
- One process per patient leads to variability, high costs, complex reimbursement mechanisms, and high impact of manufacturing failures
- Challenging to address tumor-specific MOA due to gene editing limitations

Developmental Biology

Function

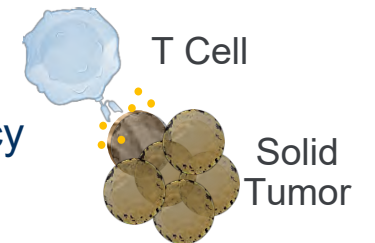
Manufacturing

Product Design



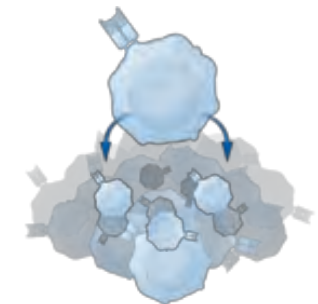
Enable unlimited T cell production from iPSCs through exquisite control of Notch Signaling

Enhance product efficacy by optimizing uniformity and potency



Improve accessibility by eliminating manufacturing constraints

Multiplexed genome engineering at the iPSC stage to overcome mechanisms of resistance in the challenging tumor microenvironment





# Controlled Notch signaling is required for T cell development

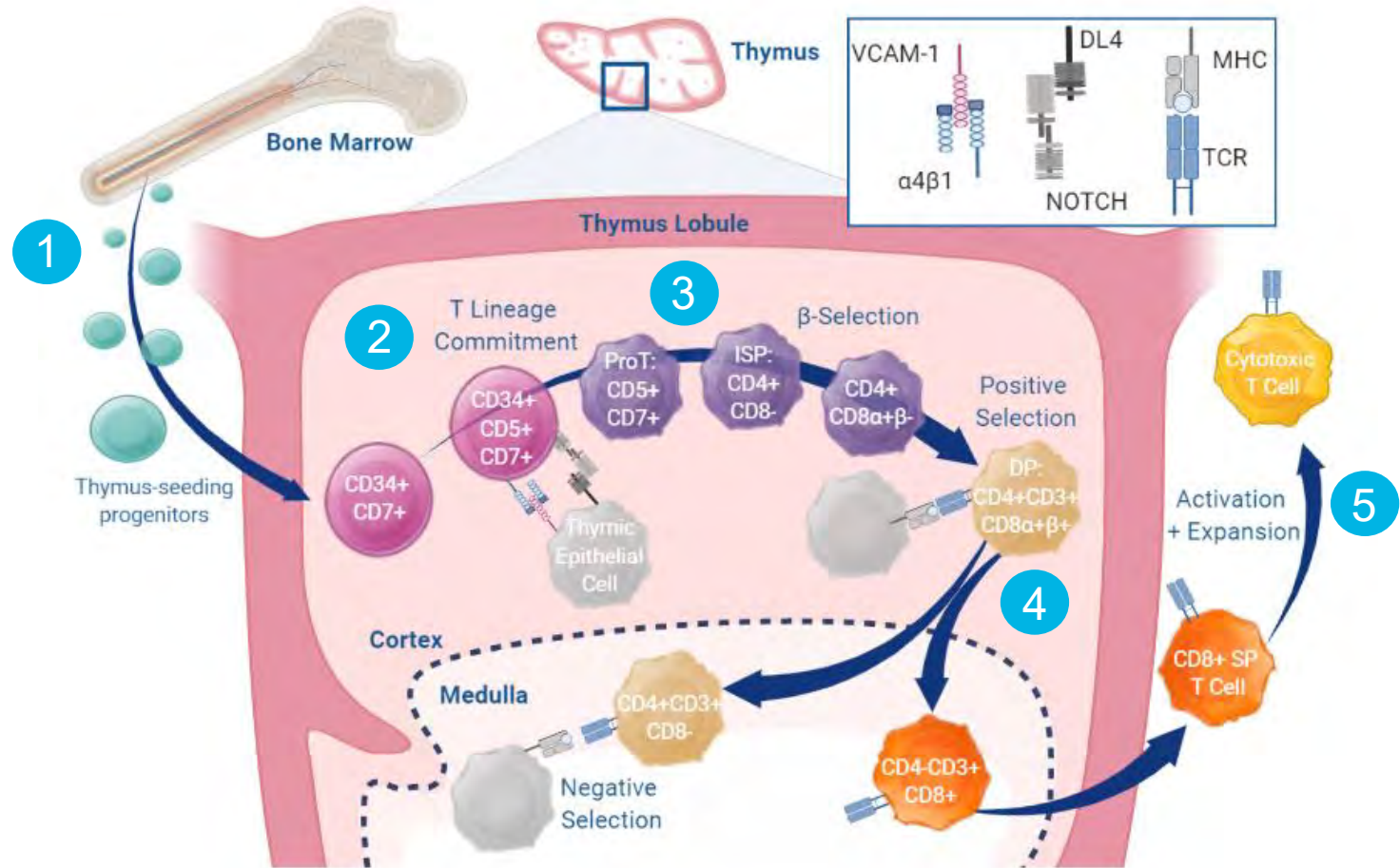
Developmental Biology

Function

Manufacturing

Product Design

Sequential, temporal and spatial development of T cells in the thymus



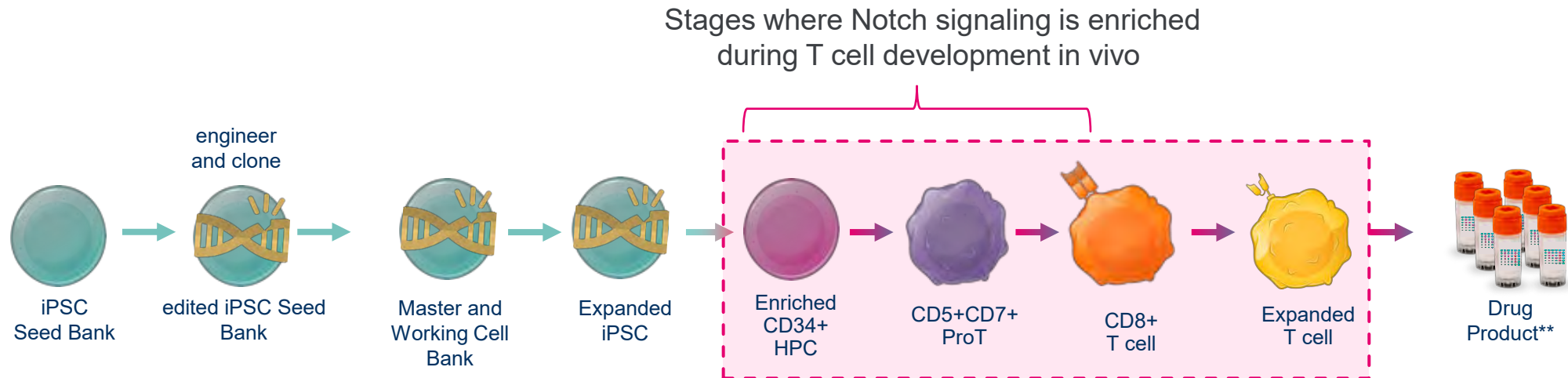
# Manufacturing process must recapitulate T cell development in vitro

Developmental Biology

Function

Manufacturing

Product Design



# Engineered 3D control of Notch signaling

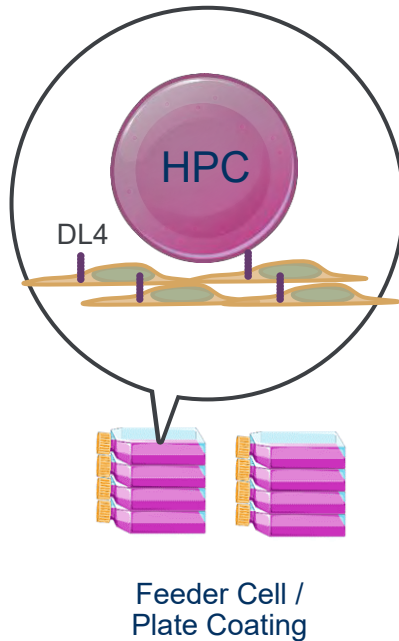
Developmental Biology

Function

Manufacturing

Product Design

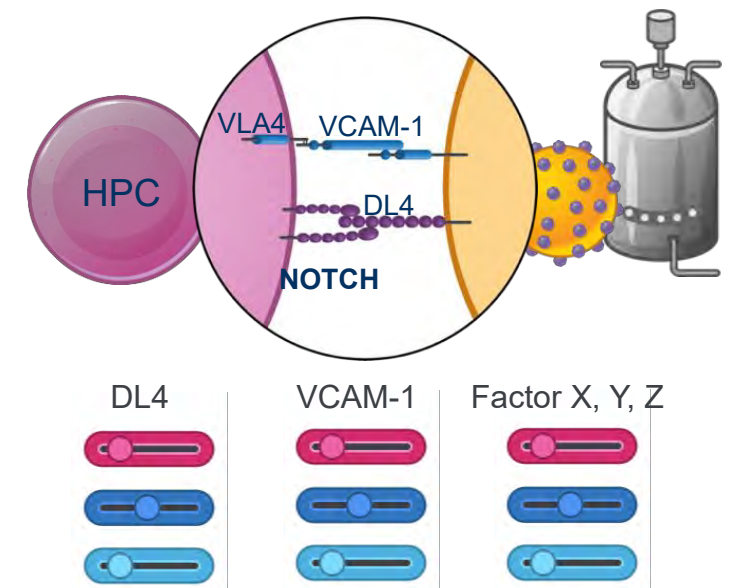
## Current Plate-Based Approaches



## Control of Notch signaling optimizes cell differentiation

- ✓ **Tunable:** Ligand concentration, surface density, timing parameters are controlled independently to control cell fate
- ✓ **Precise:** Fully defined ancillary reagents, controlled and predictable reduce variability during manufacturing
- ✓ **Predictable:** Products do not need to incorporate an additional cell line, reducing lot-to-lot variability and regulatory complexity
- ✓ **Scalable:** Fully compatible with 3D scaled manufacturing approaches to ensure supply of clinical and commercial materials

## Proprietary Engineered Thymic Niche (ETN) Bead-Based Manufacturing



# IPSC manufacturing considerations – what are we focused on?

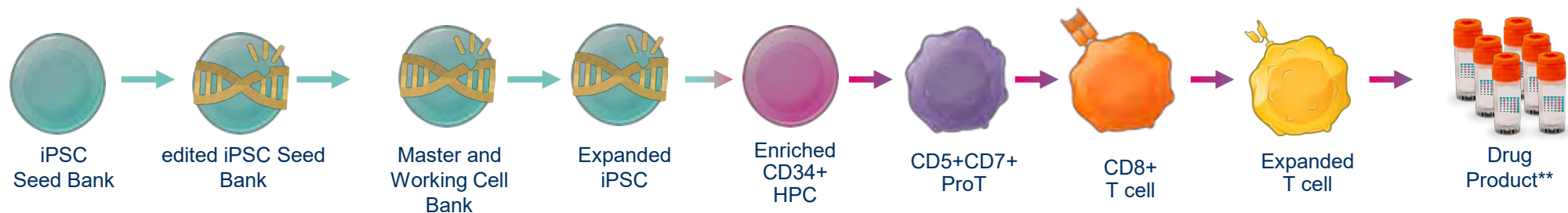
Developmental Biology

Function

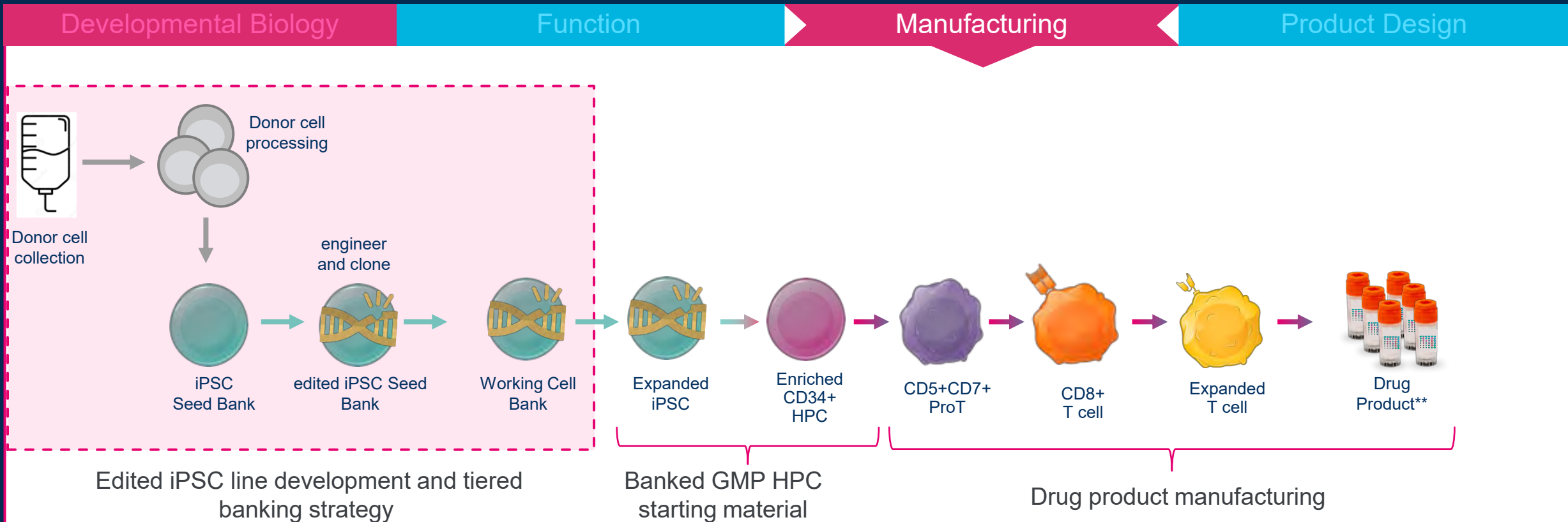
Manufacturing

Product Design

1. Starting material – high quality and fully controlled iPSC line generation
2. Tiered iPSC banks – well characterized gene editing, clone screening and 2D iPSC culture
3. Scalable iPSC expansion – STR based aggregate culture



# Manufacturing approach focuses on generation of well-characterized starting materials





# Products require early generation of custom GMP cell lines

Developmental Biology

Function

Manufacturing

Product Design

- The iPSC line is the foundation of the cell therapy product
- We invest heavily in development that is specific to the selected iPSC line
- Switching lines later in development will have cost and timeline implications
- Ideally the iPSC line will be appropriate for both clinical and future commercial manufacturing of drug product

## Donor selection criteria:

- ✓ Tissue source & donor cell type
- ✓ Sample procurement validity
- ✓ Donor eligibility testing, donor history, informed consent
- ✓ Age, gender, haplotype, blood type
- ✓ Vendor selection; fresh collection vs. frozen
- ✓ **21 CFR 1271**

## Reprogramming:

- ✓ Feasibility of selected technology
- ✓ Regulatory considerations
- ✓ GMP process development and raw materials selection
- ✓ Release assay development

## iPSC seed bank characterization:

- ✓ Cell count & viability
- ✓ Pluripotency
- ✓ STR
- ✓ Sterility
- ✓ Mycoplasma
- ✓ Endotoxin
- ✓ Karyotype
- ✓ Vector clearance
- ✓ T cell differentiation potential

# GMP iPSC lines tested for T-lineage potential and genomic stability through process

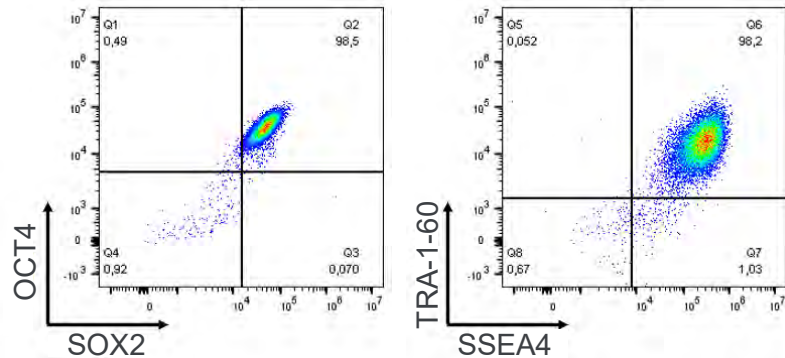
Developmental Biology

Function

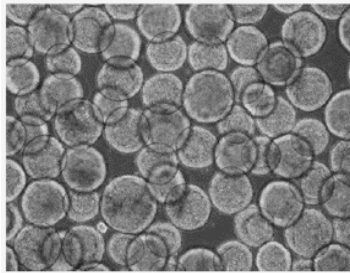
Manufacturing

Product Design

## Pluripotency Markers



## Morphology of suspension aggregates



iPSCs grown in clinical-grade 3D scaled culture format form uniform aggregates and remain highly pluripotent

## iPSC Line Release Criteria

Pluripotency	✓ Pass
Standard sterility assays	✓ Pass
Growth kinetics	✓ Pass
PSC aggregate formation	✓ Pass
Lymphoid differentiation potential	✓ Pass
Genomic stability	✓ Pass

## Clinical and Commercial Readiness:

- ✓ Donor testing and consents consistent with global regulatory requirements
- ✓ Full traceability of all manufacturing steps

# Generation of clonal, gene-edited iPSC lines

Developmental Biology

Function

Manufacturing

Product Design



iPSC  
Seed Bank

Editing

Single Cell  
Printing

Screening  
Emergent  
Clones

Selection &  
Expansion

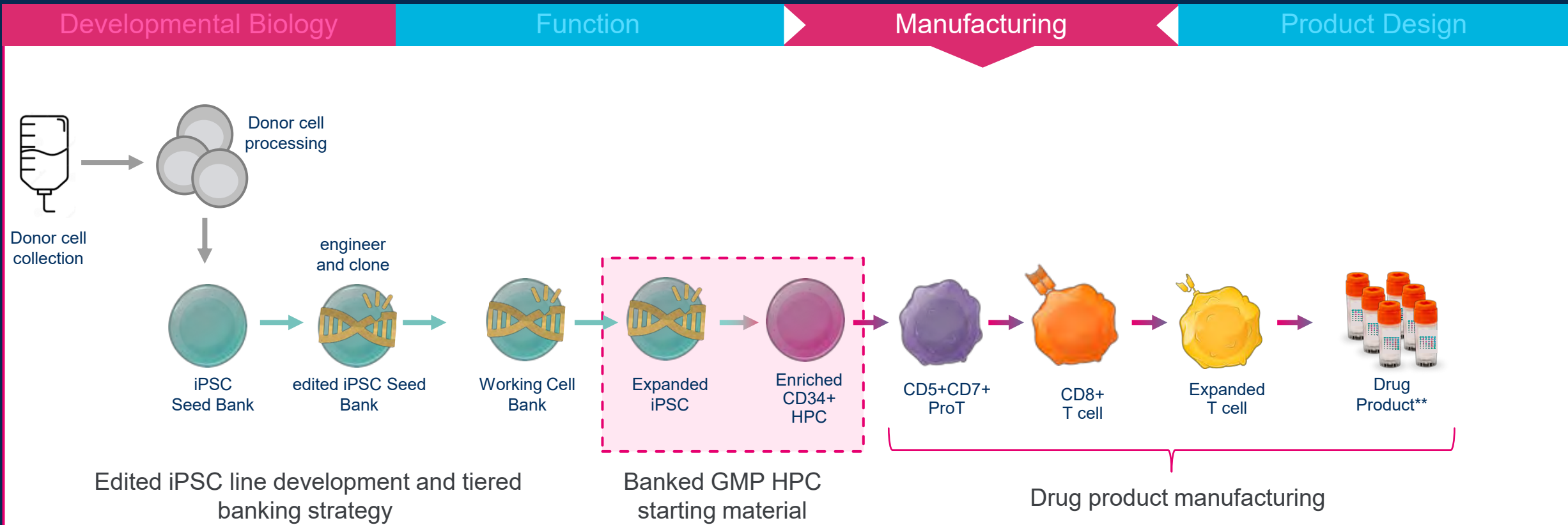
Banking

Testing



edited iPSC Seed  
Bank

# Manufacturing approach focuses on scalable iPSC expansion





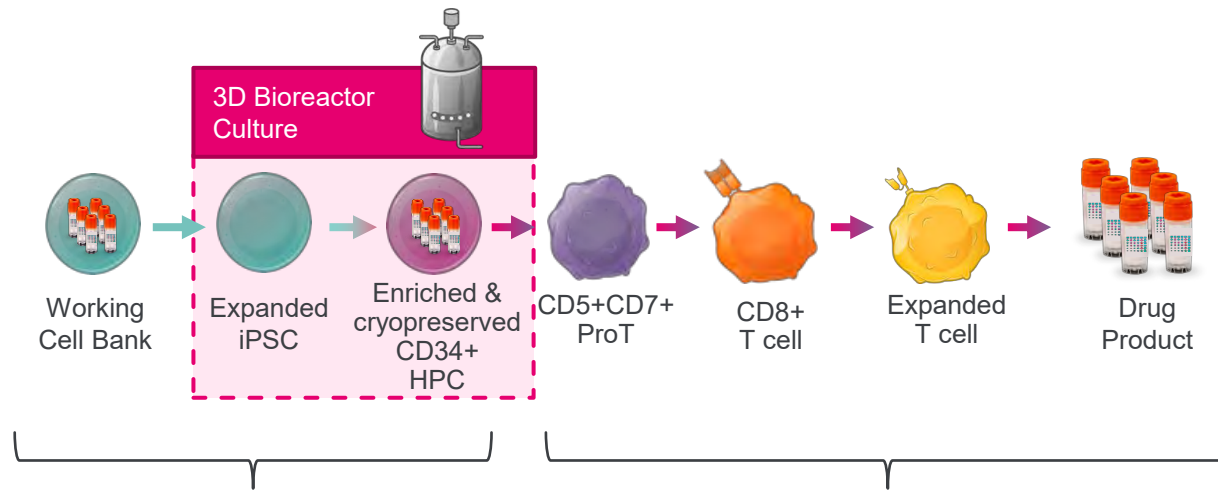
# iPSC expansion & CD34 differentiation platform enables banking of large stock of CD34 starting material

Developmental Biology

Function

Manufacturing

Product Design



Preparation of well-characterized starting materials

Drug product manufacturing

Scalable Platform Achieved

single batch of **1E9** CD34+ cells achieved, which can support **10x** 10L batches of CAR-T cells

Scalable Platform in Process Development

single 10L batch of **1E11** total cells could supply a Phase 1/2 clinical trial

Small footprint, suspension-based solutions for each stage of T cell manufacturing

- ✓ Leverage expansion potential of iPSC to generate large supply of CD34+ HPCs
- ✓ Consistent source of CD34+ starting material reduces manufacturing risk and drug product variability
- ✓ Tunable Notch signal is delivered in suspension

# STR based iPSC expansion process considerations

Developmental Biology

Function

Manufacturing

Product Design

## Control of 2D input iPSC material:

- Characterized WCB
- Choice of reagents for culture and dissociation
- Well controlled 2D PSC culture
  - Growth kinetics of line
  - PSC characterization
  - Genomic integrity



## Control and modulation of iPSCs in STR

- Choice of reagents
- Control and modulation over aggregate formation and survival
- Perfusion technology and feeding strategy
- In-process monitoring
- Culture intensification
- Aggregate dissociation
- Analytics

# **Notch** is maximizing the benefits of cell therapy by streamlining regulatory, clinical, and commercial outcomes

- **World experts in control of Notch Signaling during T cell differentiation**
  - ✓ Unparalleled expertise in developmental immunology and the role of Notch signaling during hematopoiesis
- **Optimizing cell uniformity and potency to improve T cell function**
  - ✓ Large iPSC T cell banks will enable the full characterization of drug products, so cell therapies can be treated like more traditional medicines
- **Commercially-compatible, scaled stem cell manufacturing**
  - ✓ Uniform and well characterized product to meet CMC regulatory standards and reduce clinical variability
- **Sophisticated product design**
  - ✓ Multi-edit product design to maximize effectiveness across multiple tumor types and disease areas

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➔ **End-to-end capabilities for iPSC-based therapeutics**