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Comparability Strategies for Autologous Genetically Modified Hematopoietic Stem Cells (GM-HSC)

LET'S RECODE THE STORY

Mike Havert

NASDAQ: BLUE

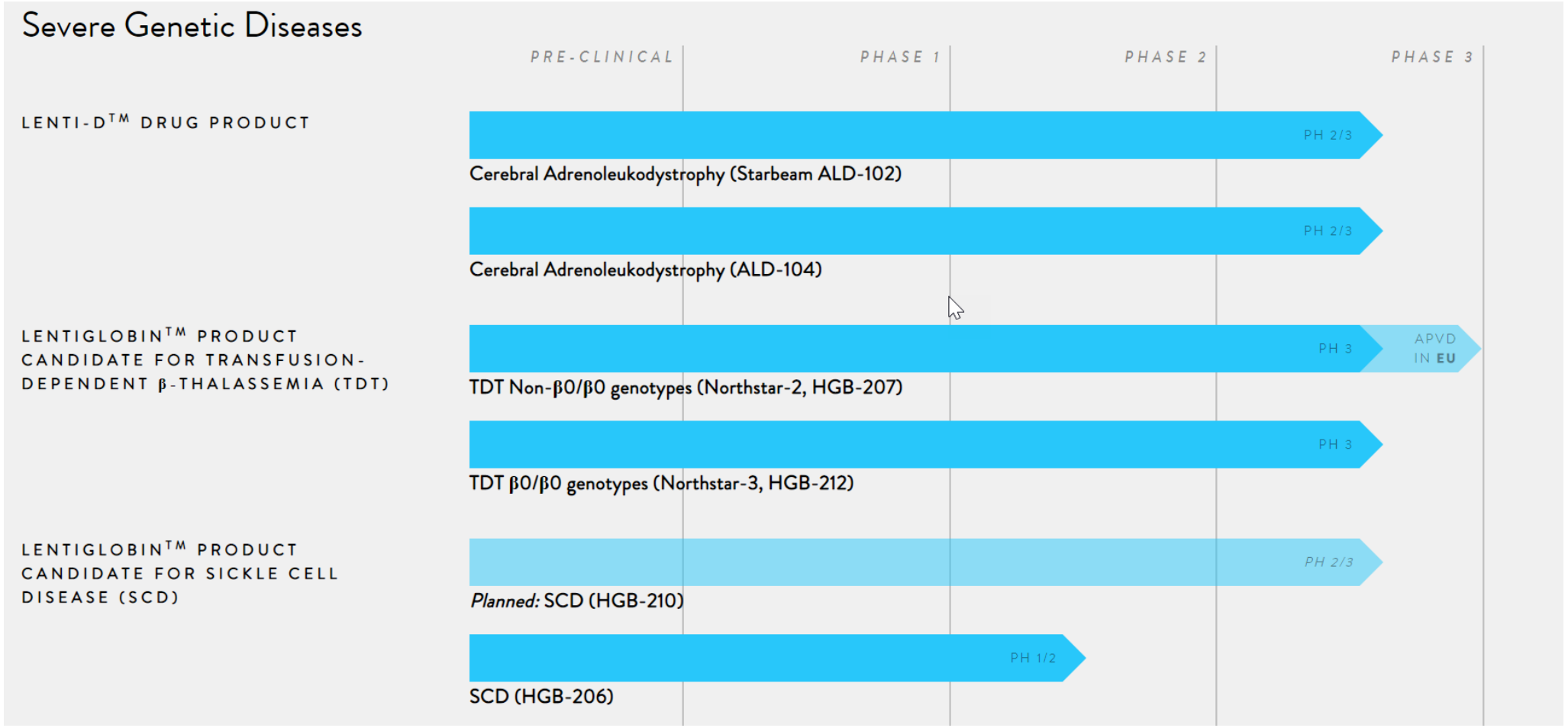
Disclosures

- Employee and stockholder - bluebird bio

Overview

- Introduce GM-HSC and product pipeline
  - Variable starting materials
  - Analytics under development
- Manufacturing changes and approaches for demonstrating comparability
  - Analytical Equivalence and Split Apheresis
  - Analytics and Manufacturing improvements

bluebird bio products and pipeline



GM-HSC treatment and manufacture overview

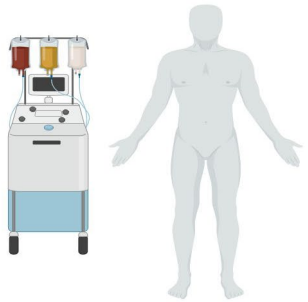
Treatment

HSC collection
Mobilization and apheresis

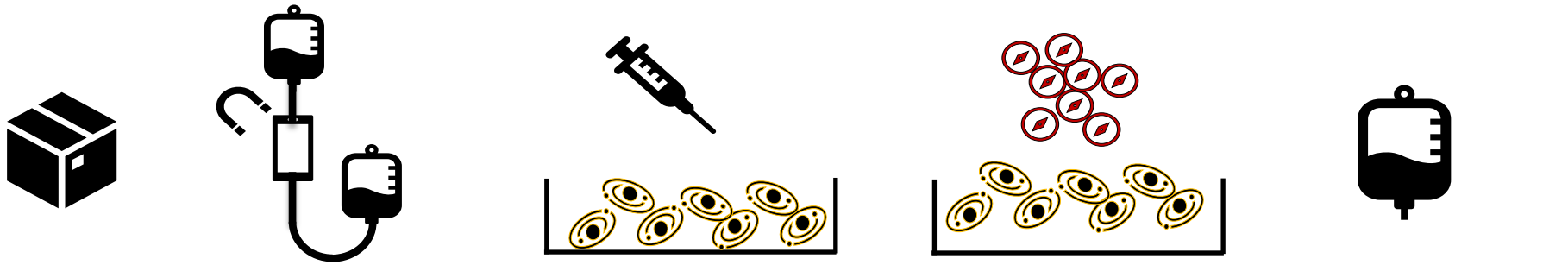
Myeloablative conditioning

Drug product infusion

GM-HSCs engraft and reconstitute blood cells



Manufacture



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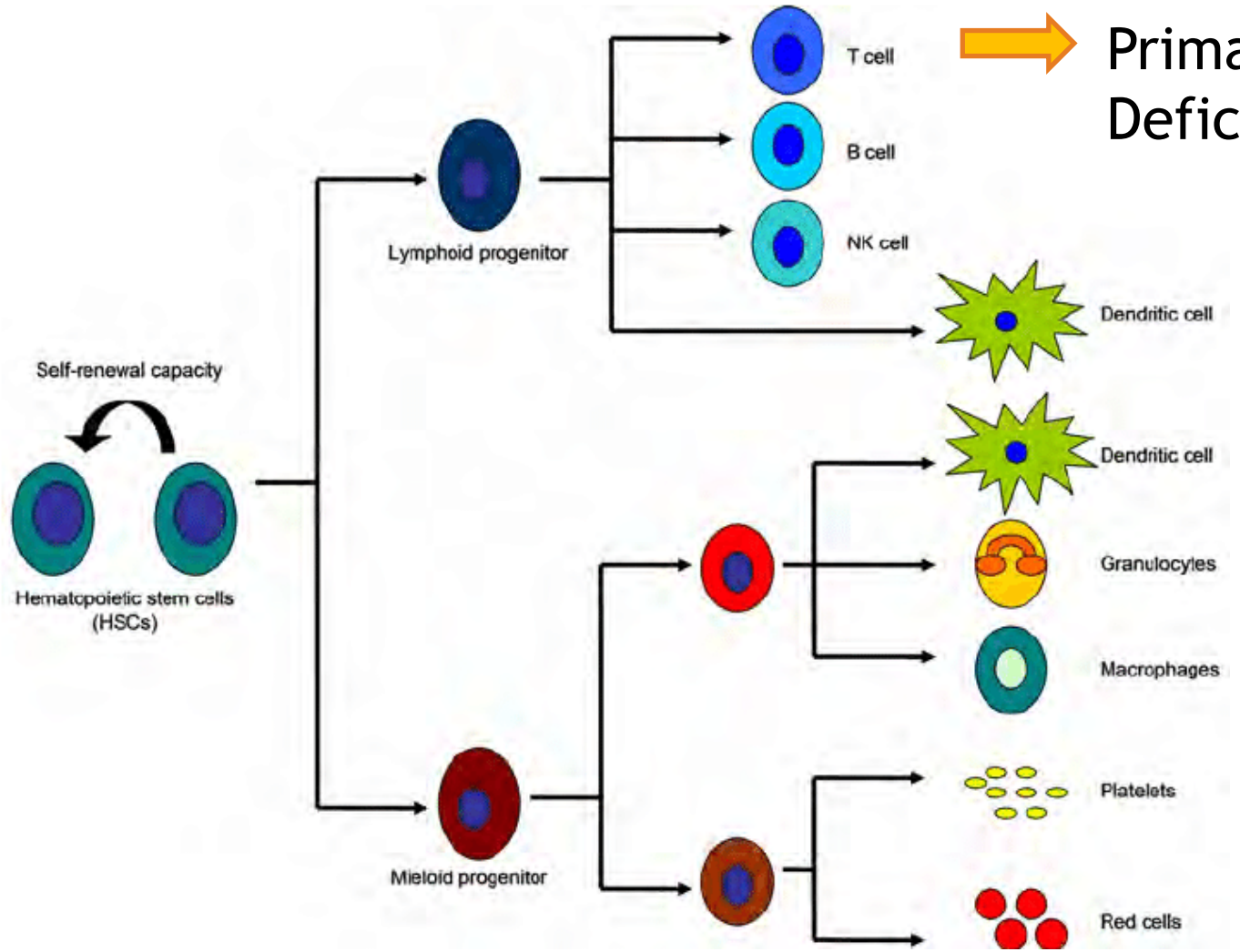
Potential treatments based on genetic modification of stem cells



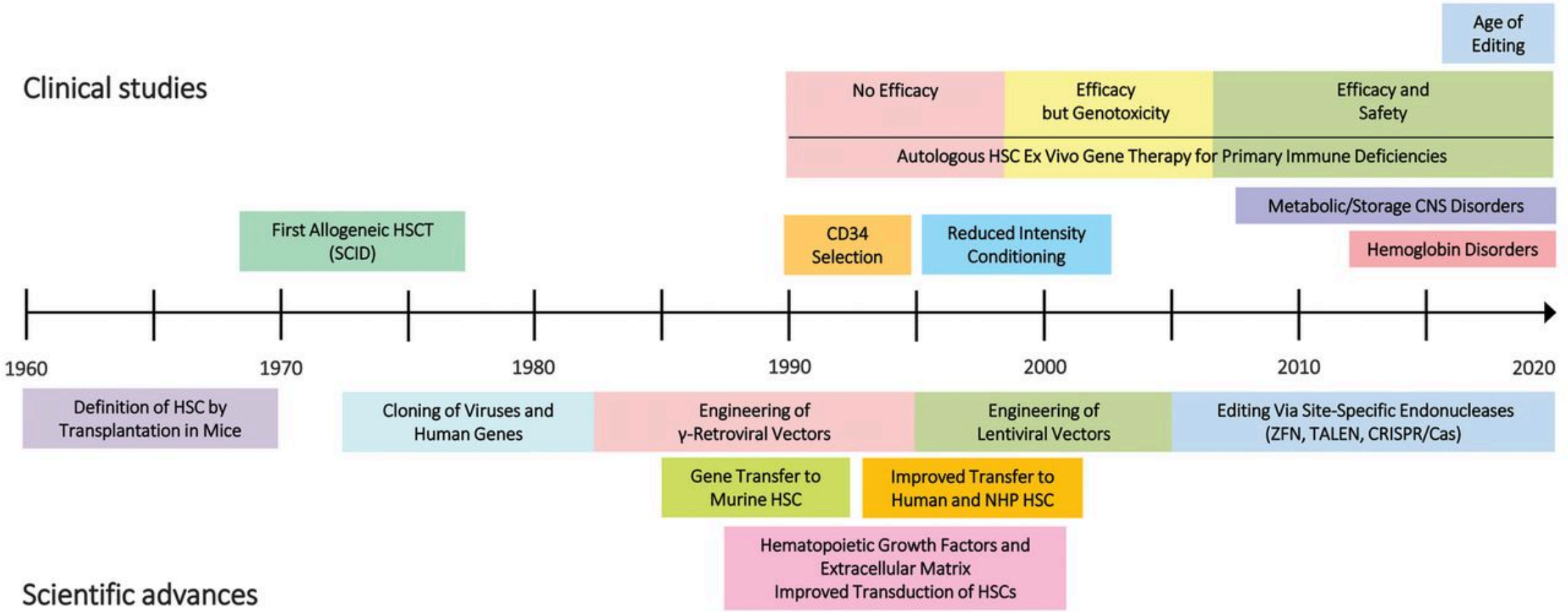
Primary Immune Deficiency

CNS disease
(Adrenal Leukodystrophy)

Heme diseases
(β-Thalassemia & Sickle Cell)

Adapted from Hematopoietic Stem Cell in Acute Myeloid Leukemia Development, Sérgio Paulo Bydlowski and Felipe de Lara Janz

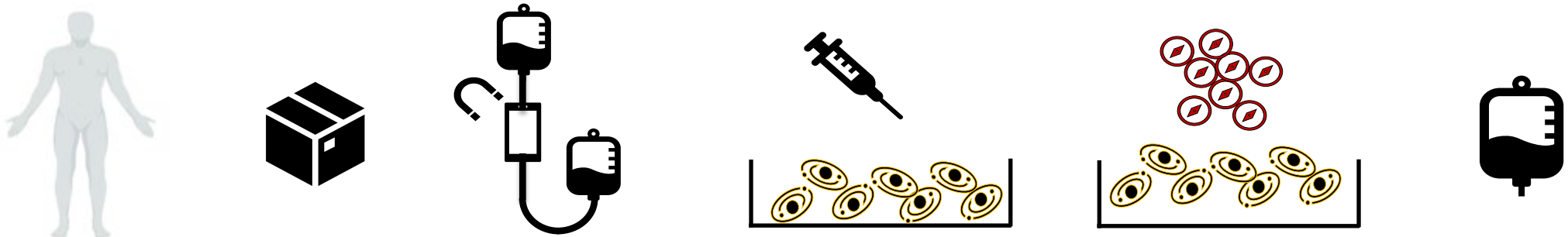
GM-HSC historical development



Cynthia E. Dunbar et al. Science 2018

GM-HSC manufacture and controls

4 days



In-process controls: Total Nucleated Cells (TNC)
%CD34+
Viable CD34+

Final product TNC
%CD34+
Viable CD34+

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Allo-HPC/cord blood cell products

INDICATIONS AND USAGE

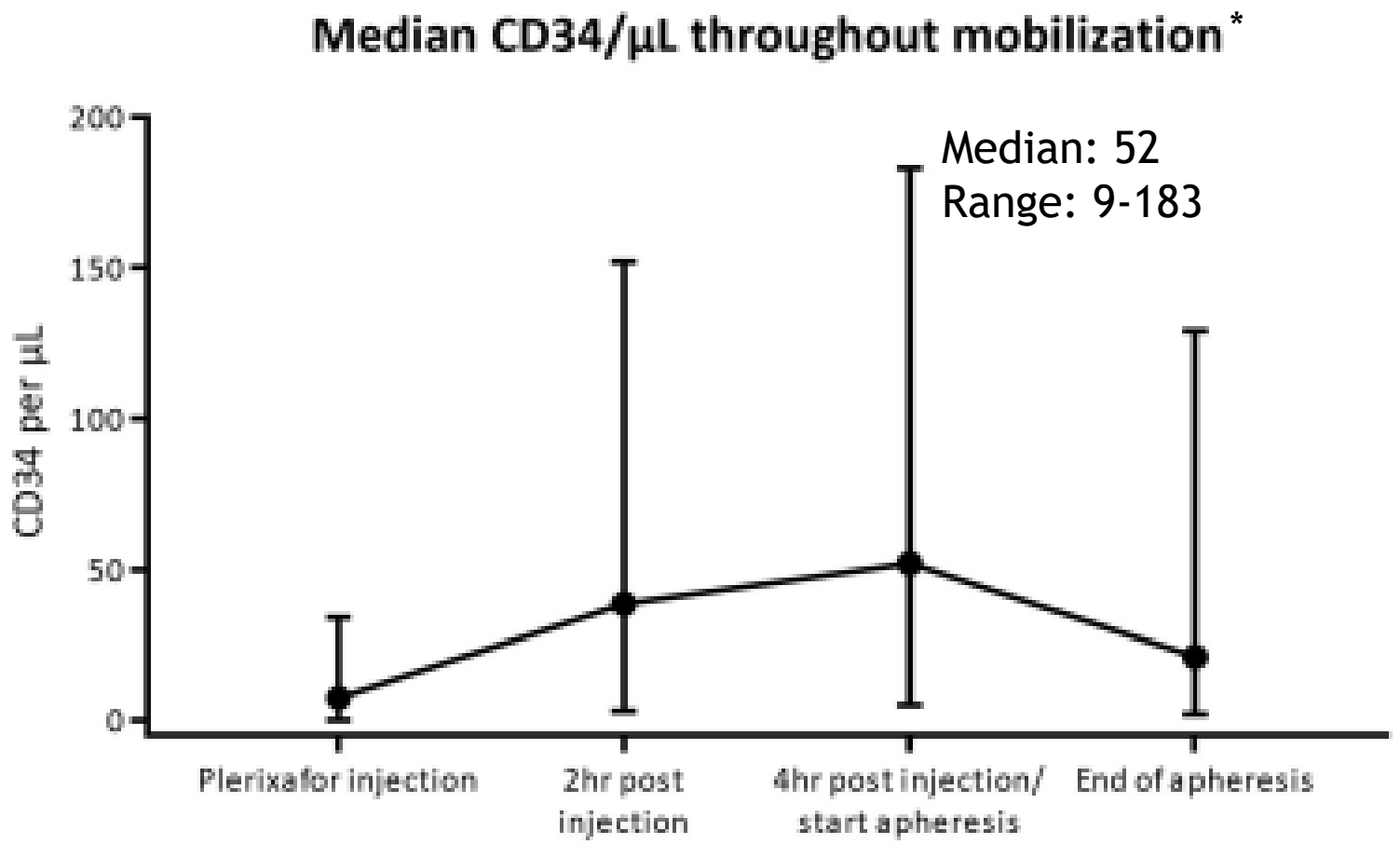
HEMACORD HPC, Cord Blood, is an allogeneic cord blood hematopoietic progenitor cell therapy indicated for use in unrelated donor hematopoietic progenitor cell transplantation procedures in conjunction with an appropriate preparative regimen for hematopoietic and immunologic reconstitution in patients with disorders affecting the hematopoietic system that are inherited, acquired, or result from myeloablative treatment (1)

Table 2: Testing of HPC-C[1]

Product Characteristics[2]	Testing	Sample (Type and Timing)	Results of Product Testing
Safety	Infectious diseases – Testing Required. (21 CFR 1271.45 through 1271.90)	Maternal peripheral blood obtained within 7 days of cord blood collection – Type and Timing Required. (21 CFR 1271.80(a) and (b))	All tests negative except non-treponemal test for syphilis when confirmatory test is negative. (Cytomegalovirus (CMV) results are recorded.)
			CMV – Report
	Sterility – Bacterial and fungal cultures – Testing Required. (21 CFR 211.165(b), and 21 CFR 610.12)	HPC-C (pre-cryopreservation) **	No growth
	Hemoglobin	Cord blood*	No homozygous hemoglobinopathy
Purity and Potency[3]	Total nucleated cells (TNC)	HPC-C (pre-cryopreservation)	≥ 5.0 x 10^8 TNC ***/ unit HPC-C
	Viable nucleated cells	HPC-C (pre-cryopreservation)	≥ 85% viable nucleated cells
	Viable CD34+ cells (flow cytometry)	HPC-C (pre-cryopreservation)	≥ 1.25 x 10^6 viable CD34+ cells ****/ unit HPC-C
Identity	Human leukocyte antigen (HLA) Typing	Cord blood	Report
	Confirmatory HLA typing	Attached segment of HPC-C	Confirms initial typing
	Blood Group and Rh Type	Cord blood	Report

[1] *Testing, Sample (Type and Timing), and Results are recommended unless specifically noted as required.*
[2] *The PHS Act requires a demonstration that the product is safe, pure, and potent.*
[3] *Other purity and potency assays may be considered under the BLA.*
* *Cord blood = a sample of unmanipulated cord blood. A red cell sample or other cord blood aliquot obtained after volume reduction may be used for testing with appropriately validated reagents or test systems.*
** *Sample may be obtained before or after addition of the cryoprotectant.*
*** *Based on 20 kg recipient, a target dose of ≥ 2.5 x 10^7 nucleated cells/kg and ≥ 70% post-thaw recovery = 1.7 x 10^7 nucleated cells/kg.*
**** *Based on CD34+ cells ≥ 0.25% of TNC prior to freezing.*

Variability of CD34+ cell concentration



*Uchida et al. Haematologica 2020 "Safe and efficient peripheral blood stem cell collection in patients with sickle cell disease "

bluebird bio manufacturing has changed over time

- Facility (CMO)
- Process
- Materials
- Intermediates
- Specification
- Analytical procedures
- Excipients

How do we know the product is still safe?

How do we know the product is still effective?

How do we know the products are comparable?

bluebird bio manufacturing has changed over time

- Facility (CMO)
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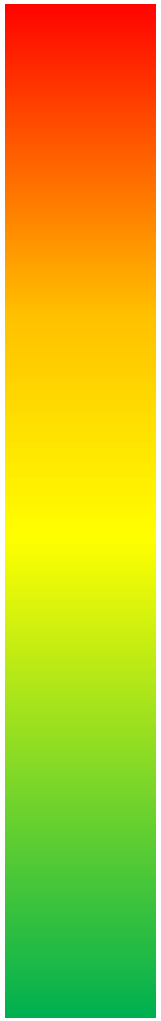


High Risk

Low Frequency

Moderate Risk

Low Risk

High Frequency

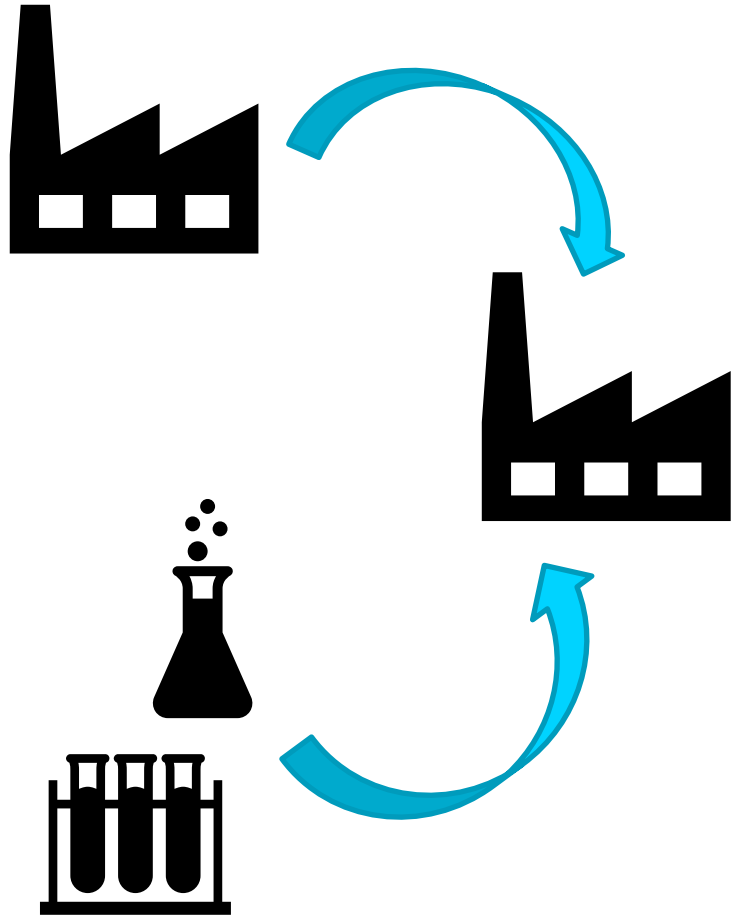
Mitigation

Technology Transfer

Change Control

Technology transfer

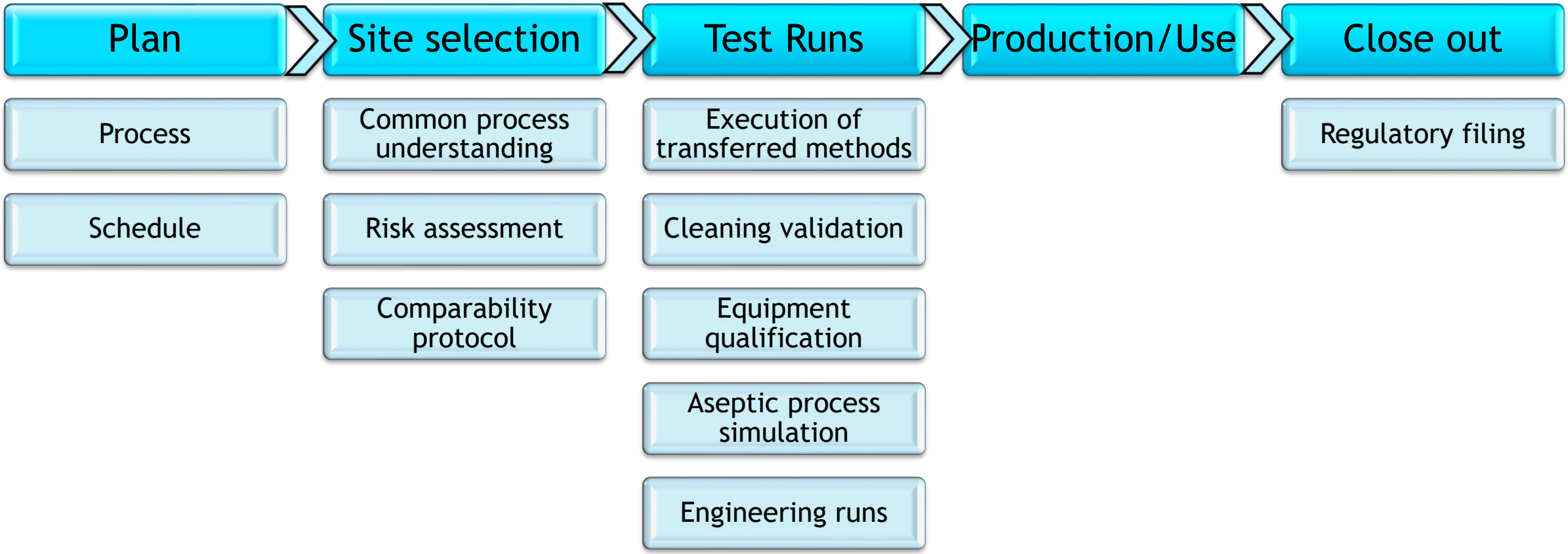
- Transfer of manufacturing processes and/or analytical procedures between facilities or laboratories
- Technology transfers take the outputs of process or method development activities and transfer the knowledge to a different location where a process or analytical procedure will be operated.



ISPE Good Practice Guide: Technology Transfer 3rd Edition

WHO guidelines on transfer of technology in pharmaceutical manufacturing

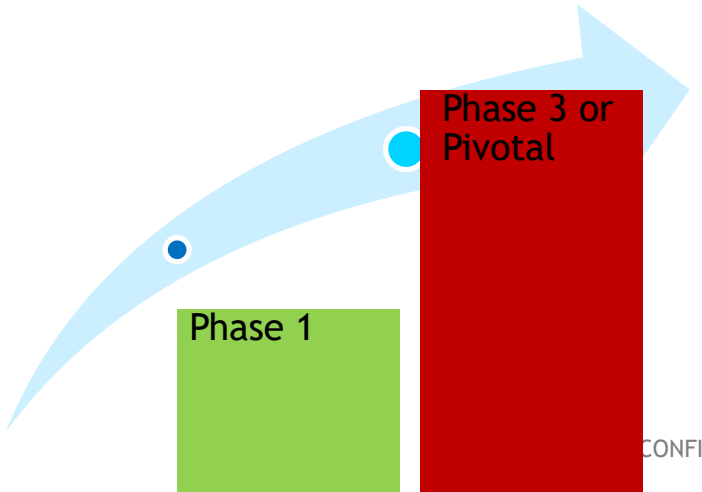
Technology transfer overview



Regulatory standards for evaluating risk under IND

- CMC changes that impact Safety - 21 CFR 312.42(b)(1)(i)
  - FDA may place a proposed or ongoing Phase 1 investigation on clinical hold if it finds that: Human subjects are or would be exposed to an unreasonable and significant risk of illness or injury

- CMC changes that impact Efficacy - 21 CFR 312.42(b)(4)
  - FDA may place a proposed or ongoing investigation that is not designed to be adequate and well-controlled
  - Applies to all trials, but usually used for phase 3/pivotal



Types of comparability exercises

- Analytical
- Nonclinical / animal model
- Clinical

Analytical comparability considerations

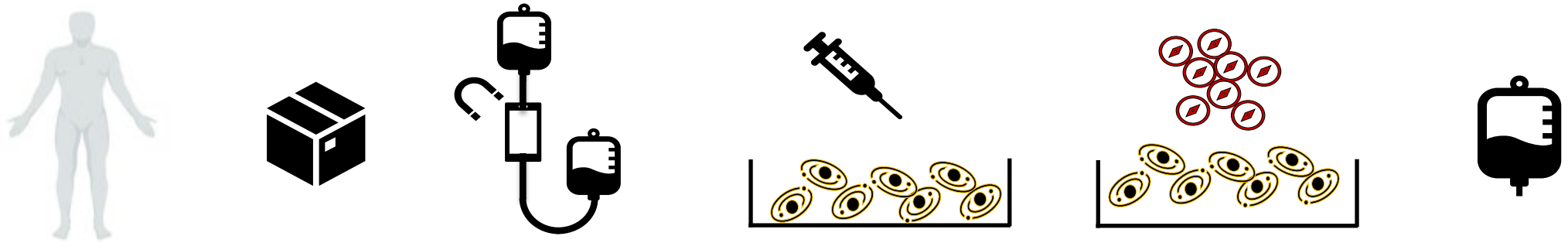
ICH Q5E Comparability of Biotechnological/Biological Products Subject to Changes in Their Manufacturing Process

- What samples?
  - Pre-change vs Post-change
    - Full scale vs lab scale manufacturing runs
    - Retrospective or prospective?
    - Lot retains
    - Split apheresis
  - Sample size – how many?
- What are analytics?
  - Lot release (validated?)
  - More than release
    - Characterization tests
    - In process test
    - Stability data
- Statistical assessment regarding equivalence
  - Demonstrating sameness
  - Setting study criteria

Split Apheresis comparability

- Split starting material eliminates person-to-person variability
- Healthy donor
- Test panel (no potency) – can restore gene expression to cells that already have it
  - Total nucleated cells (TNC)
  - %CD34+ cells
  - Viable CD34+ cells

Split Apheresis comparability



	Collection	CMO #1	Wash and CD34+ Cell Selection	Culture	Transduction	Cryopreserved Drug Product
TNC*	4.6×10^{10}	4.7×10^{10}	6.6×10^{8}	5.7×10^{8}	5.0×10^{8}	4.5×10^{8}
%CD34+	1.5		95	98	99	96
TNC	4.7×10^{10}	4.4×10^{10}	4.3×10^{8}	7.0×10^{8}	5.3×10^{8}	5.1×10^{8}
%CD34+	1.5		97	98	92	98

	Collection	CMO #2	Wash and CD34+ Cell Selection	Culture	Transduction	Cryopreserved Drug Product

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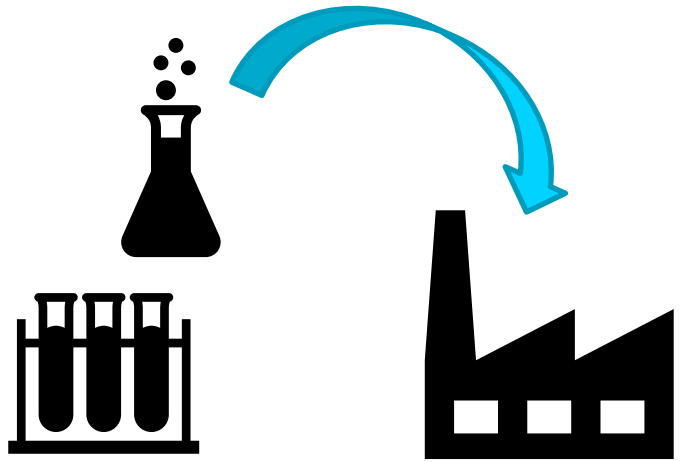
* TNC = Total nucleated cells

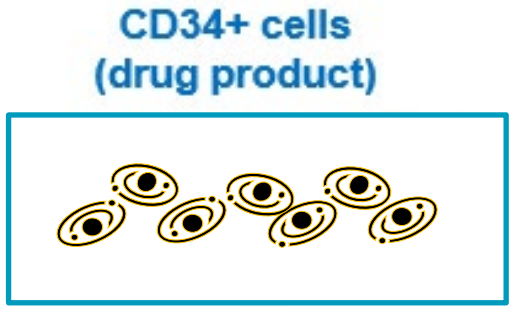
Analytics and manufacturing improvements

- Develop analytics and bioanalytics
- Optimize manufacturing process in lab
  - Improved product quality
  - Pre-change ≠ post-change*
- Technology transfer to CMO
- Update CMC section IND
- Clinical studies



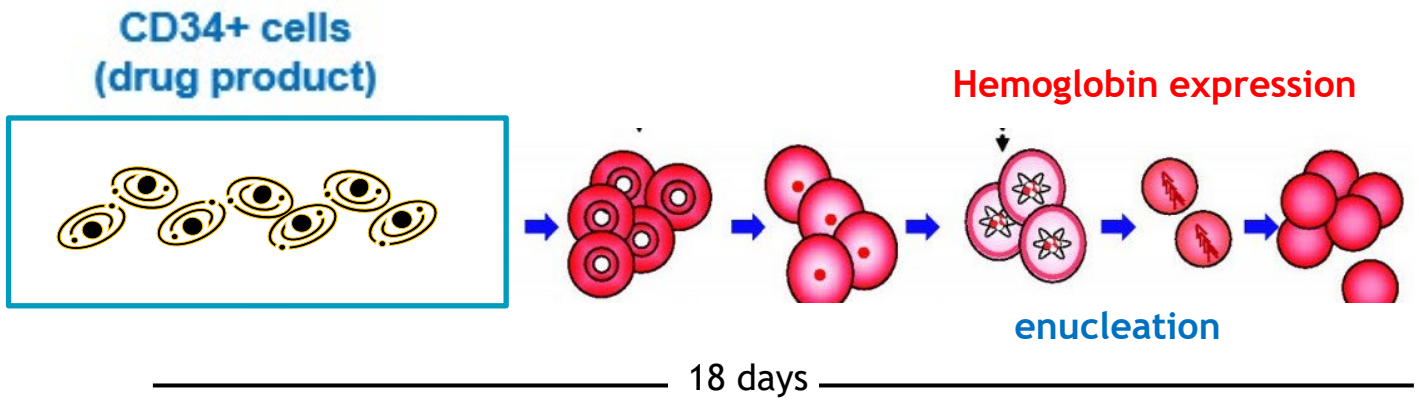
***ICHQ5E** - When pre- and post-change products are not comparable, please consult the appropriate regional regulatory authority

Analytics…



- Not possible to assess functional activity in final drug product (CD34+ cells) if transgene is not expressed

Analytics…



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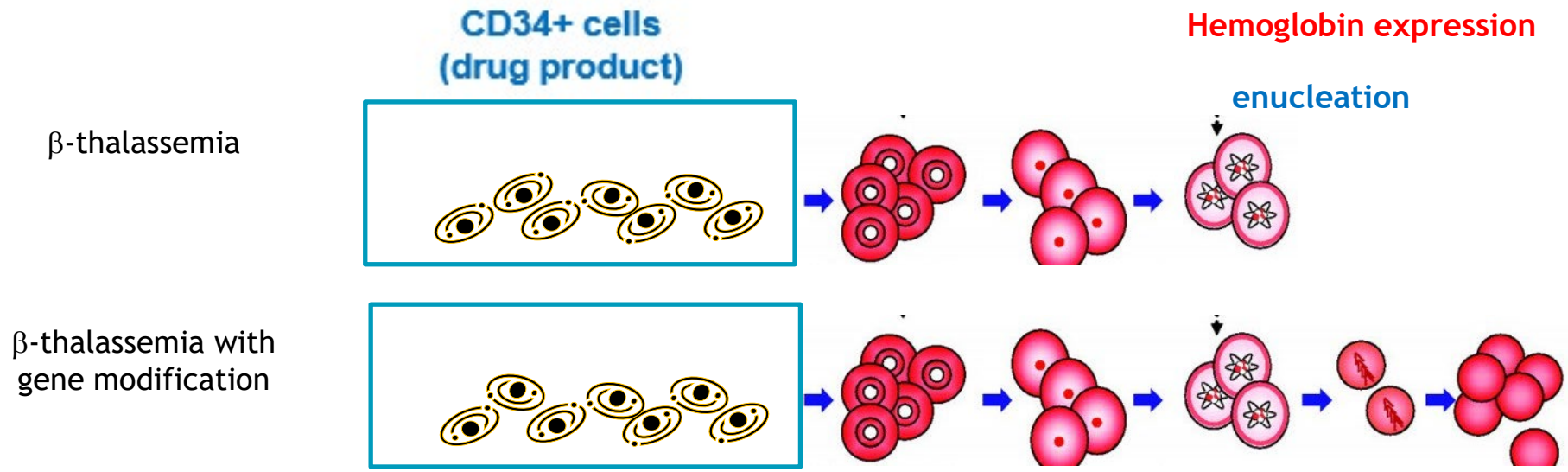
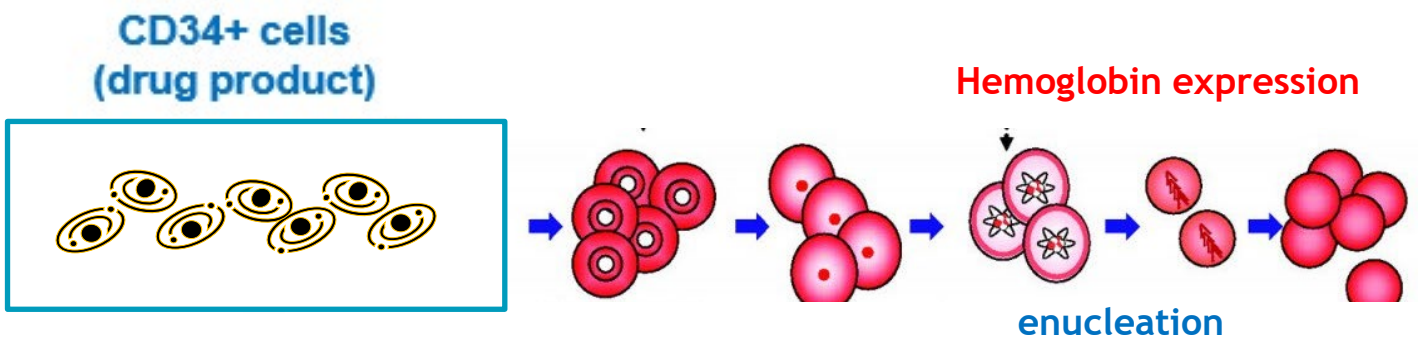
Analytics…



- Gene modification corrects arrest at enucleation step in β-thalassemia
- Potency can be measured as a relative increase in % enucleated cells

Image adapted from: hubpages.com/education/General-Considerations-In-Hematology-Blood-Formation-In-Erythropoiesis

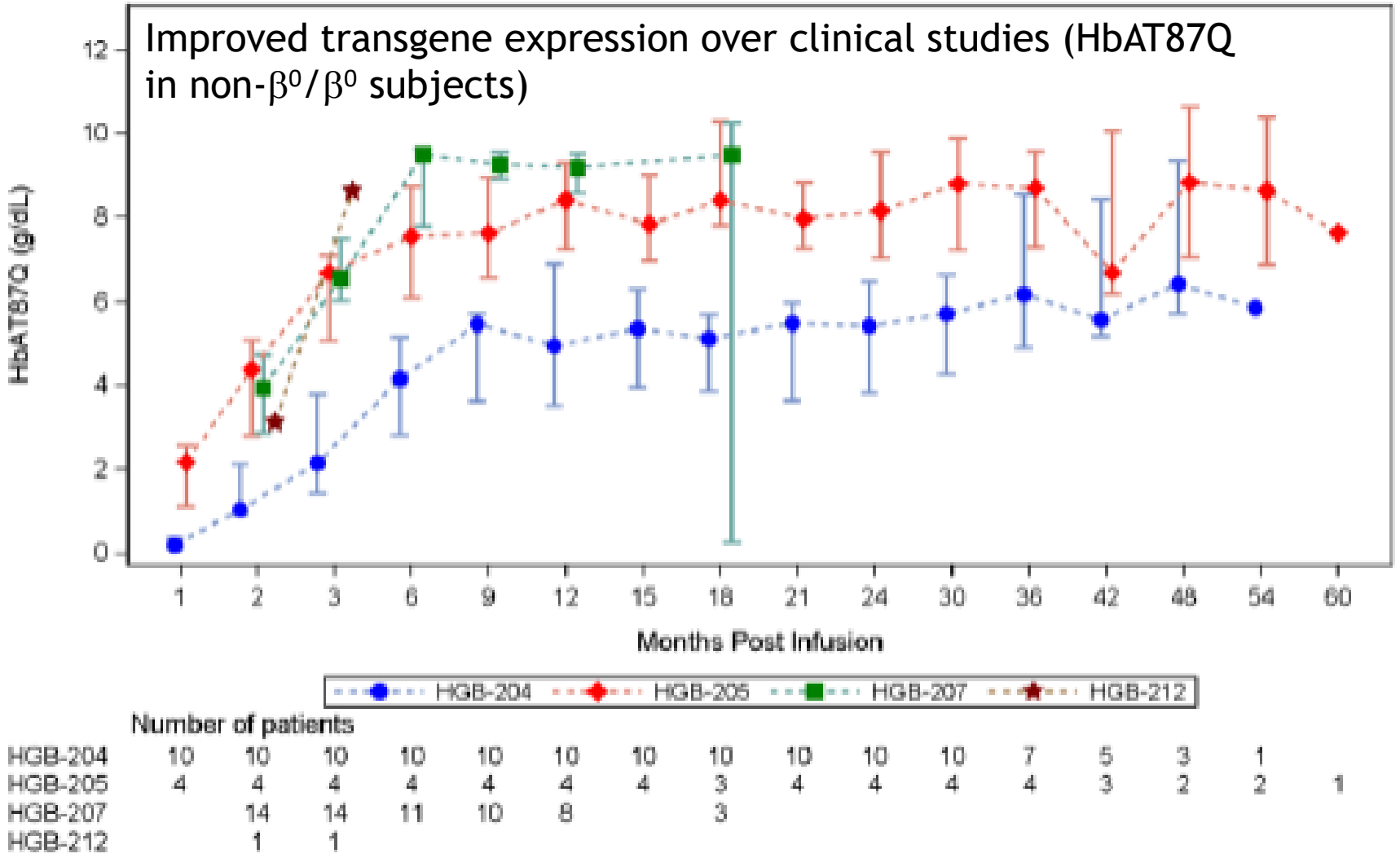
Analytics…



Protein expression

Number of transgene copies inserted (Vector Copy Number - VCN)
Percent cells with transgene (%LVV+)

Analytics and manufacturing improvements in clinical studies



Zynteglo EPAR – public assessment report 14-Feb-20

Summary

- Technology transfer
- Invest in analytics
  - Potency and stability indicating assays
  - Bioanalytics and data science too
  - Less variability is better
- Minimize manufacturing variability (split aph)
- Get clinical experience (introduce changes early)
- Learn from mistakes
  - Small scale models to de-risk

The bluebird flock

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